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THE RELATIONSHIP BETWEEN VISCOSITY, TOTAL PROTEIN CONTENT, ASH CONTENT, AND BAKING STRENGTH OF EXPERIMENTALLY AND COMMERCIALLY MILLED FLOURS FROM WESTERN CANADIAN HARD RED SPRING WHEAT

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Introduction

It is now generally recognized by cereal chemists that the baking properties of wheat flour are determined by at least two factors; (1) gas production in, and (2) gas retention by the dough. Gas production is influenced by substances present in or added to the dough, which act either as yeast stimulants or as yeast foods, and include diastatic enzymes, fermentable sugars, salts, nitrogenous materials, etc. Most of the factors influencing gas production are more or less controlled by the miller or baker, and are probably secondary in importance to the factors influencing gas retention.

The most important factor influencing gas retention is the character of the gluten present in the dough. It is the presence of two proteins, gliadin and glutenin, which make it possible for a wheat flour dough to contain any gas at all. Many investigators have demonstrated that total protein content is highly correlated with baking strength. Although total protein content is not identical with gluten content, the two are usually (in sound flours at least) highly correlated, and as total protein content is readily estimated by the Kjeldahl determination, it is usually taken as representing gluten content. Total protein content, then, is generally recognized as one of the most important measures of flour strength. Consequently, millers buy and bin their wheat, compound their mill mixes, and sell their flour on a protein basis.

Total protein content, however, is probably not the lone determining factor. Many exceptions, based on the fact that when a series of flours is baked the results are not always as forecasted by protein con-

tent alone, indicate that other factors beside protein content may also play a leading part in determining flour strength. These exceptions may be the result of a baking test that does not give the proper information, but it is also very probable that the proteins in different samples of wheat have different *make-ups* and are probably of different *qualities*. In cases where high protein flour is apparently of low strength, it is said that the proteins are of poor quality, and vice versa.

Many devices have been constructed in an effort to measure gluten quality, none of which have been wholly successful. Probably the principal reason for this is, that these measures of quality have been referred back to the baking test, and as this test differs and often gives contradictory results on the same sample of flour in different laboratories, there can be little agreement in the conclusions regarding the relation of estimates of quality. Many of the devices require that the gluten be first washed from the flour—a procedure probably giving inaccurate and misleading results. With some instruments, gluten quality is not the only factor measured.

Several investigators, looking at the situation from the chemical point of view, have attempted to demonstrate that differences in quality are due to differences in the chemical composition of the proteins. It is beyond the scope of this paper to give a complete review of the work that has been done to date on this subject. Suffice it to say that many theories were suggested and later discarded, until in 1908, with the development of the chemistry of substances when in the colloidal state, Wood and Hardy, and others, introduced the idea that protein or gluten quality was not due to differences in chemical composition alone, but was related to the physical properties of the proteins.

Flour is a more or less dry, granular powder. When water is added, a dough or suspension is formed, depending on the amount of water added. Part of the water exists as free water, or is merely enmeshed or adsorbed on the surfaces of the starch granules, and part of it is imbibed by the two proteins, gliadin and glutenin, causing them to swell and take the form of a colloidal sol or gel, in a manner similar to the action of gelatin, albumin, or casein with water. It can be demonstrated that the proteins of different flours imbibe different amounts of water, and also that the rate of imbibition is different with different samples.

According to this physico-chemical theory, the strong or high quality proteins imbibe the greatest amount of water, and conversely, the weak or low quality proteins imbibe a lesser amount. In ordinary water suspensions, the proteins imbibe about twice their weight of water, and become, when the starch is washed free, an elastic, rubber-like mass which is called wet gluten. The differences in hydration

capacity (or amount of water imbibed) is too small, and the method of washing the gluten free from the flour is too inaccurate to be of use as a means of determining the hydration capacity directly. However, if the gluten masses are allowed to stand in dilute acid solutions, they imbibe considerably more water, and differences in hydration capacity become more readily apparent. The maximum imbibition occurs at a pH of about 3.

As a result of the imbibition of water, colloidal systems of this type become more or less viscous. Resistance to flow, or viscosity, as it is called, may be measured either by measuring the time required for a certain amount of suspension to flow through a capillary tube, or by means of a torsional viscosimeter.

Lüers (1919, 1920) and Ostwald (1919) were the first to examine, systematically, the viscosity of flour-in-water suspensions. Later, Sharp and Gortner (1923) demonstrated that viscosity values are highly correlated with the hydration capacity of wheat flour proteins.

If the proteins with the greatest hydration capacity are the highest in quality, then the relative viscosity values of flours, when suspended in acid solutions, should be a measure of baking quality, and other things being equal, a measure of baking strength.

PART I

Experimental

Material

As part of the regular yearly survey of the wheat from our territory, a number of samples of wheat from the western and northern sections of Saskatchewan, as well as from eastern Alberta, were milled and baked.

As viscosity determinations are made in conjunction with other routine tests, data on a large number of flours of varying characteristics became available, from which a study of the viscosity test can be drawn. After all analyses and baking data were completed the data were examined statistically in order to ascertain what relationship, if any, existed between viscosity, protein content, and baking strength. Studies were made on samples from the 1930 and 1931 crops.

Methods

The Viscosity Determination: The viscosity readings were obtained on a Wallace and Tiernan torsional type viscosimeter. The method consisted of allowing a suspension of 15 gms. (moisture free basis) of flour to stand in 100 cc. of distilled water for 30 minutes at a temperature of 80° F. At the end of this period, one drop of caprylic alcohol

(to break the foam) and 5 cc. of N/1 lactic acid were added, and the viscosity immediately measured.

Since the ash content of the experimentally milled flours studied did not vary greatly, leaching with distilled water was not attempted.

The Baking Test: The formula and method used in baking were as follows:

Flour.....	340 gms.
Yeast.....	20 gms.
Sugar.....	15 gms.
Salt.....	5 gms.
Lard.....	1 teaspoonful
Water.....	enough for proper absorption

The dough was mixed in a Hobart mixer and allowed to ferment according to the following schedule:

First punch.....	45 minutes
Second punch.....	35 minutes
To pans.....	10 minutes
To oven.....	variable (about 60-70 minutes)

After baking, the loaves were measured for loaf volume and scored for baking quality. Five descriptive terms—excellent, very good, good, fair, and poor—were assigned, according to the general appearance of the loaf. This method of scoring is the regular method used in our laboratory for experimentally milled flours. It does not lend itself very readily to mathematical treatment, due to the fact that no numerical values are given and it is necessary to assume that all flours in the group described are equal in value when determining coefficients of correlation. Baking scores also take into consideration color, which is not significant when dealing with strength, and for this reason loaf volume is often taken as a measure of strength. Both values, however, were used in this study.

Correlations from the 1930 Crop Samples

For the wheats of the 1930 crop, and grading 1, 2, and 3 Northern, the coefficient of correlation between loaf volume and baking score was $+0.741 \pm 0.021$. The correlation was much lower for the lower grades of wheat and for the samples of the Garnet variety, mainly on account of the influence of the color score, which is of more importance with these samples.

It was thought that the lower grades of wheat, and wheat of the Garnet variety, might have different characteristics than the wheats grading 1, 2, and 3 Northern. Therefore, the data were segregated into four parts: Wheats grading No. 1, 2, and 3 Northern were grouped as Series I; wheats of grade No. 4 as Series II; wheats grading No. 5 and 6 as Series III; and the Garnet samples, which graded No. 2 and 3,

as Series IV. Most of the 1930 crop graded high and it was difficult to obtain many samples of the lower grades. However, it is believed that enough samples were tested to give an indication, at least, of what might be expected from a larger series.

The data are presented in the scatter diagrams shown as Figures 1 to 4. Coefficients of correlation were also calculated and are shown in Table I. The coefficients of correlation under the heading r_{xy} are a measure of the closeness of the relationship of any two variables under consideration. They were calculated from frequency tables prepared from the individual values, which were too lengthy to include, by employing the formula:

$$r_{xy} = \frac{\sum \frac{xy}{n} - \bar{x}\bar{y}}{\sigma_x \sigma_y}.$$

Where the samples were few, the significance of the correlation coefficients were discovered by means of the value t , calculated from the formula as given by Fisher (1930)

$$t = \frac{r}{\sqrt{1-r^2}} \cdot \sqrt{n-2}.$$

From the values of t and P , the probability of the correlation arising from random chance was determined from Fisher's (1930) tables. Where P equals 0.01 the correlations are considered highly significant. In a series containing a large number of samples, the significance of the correlation may be determined from the probable error.

Series I

This series consisted of 209 samples of wheats of the 1930 crop, grading No. 1, 2, and 3 Northern. The coefficients of correlation were all significant, and indicated that total protein and viscosity values are about equal in value in predicting either loaf volume or baking score as obtained by the method used. The values are similar to those obtained by other investigators, when using the same type of baking test. The correlation between protein content and the viscosity values is significant and high, as can be seen in the scatter diagram, Figure 1.

Series II

In spite of the fact that the samples grading No. 4 were damaged by frost, etc., and contained some immature kernels, the coefficients were not greatly different from those obtained with the undamaged wheats studied in Series I. In all, 39 samples were tested, 4 of which were, for some unknown reason decidedly out of line in all of the com-

TABLE I
STATISTICAL CONSTANTS FOR THE SERIES OF EXPERIMENTALLY MILLED FLOURS
OF THE 1930 AND 1931 CROPS

Number of samples	Factors correlated	Coefficient of correlation	P.E.	t	P	Value of r_{xy} at $P=0.01$
1930 Crop						
Series I						
Grades No. 1, 2, and 3 Northern						
209	Viscosity and loaf volume.....	+0.431	±0.038			
209	Protein content and loaf volume.....	+0.454	±0.037			
209	Viscosity and baking score.....	+0.517	±0.034			
209	Protein content and baking score.....	+0.507	±0.036			
209	Protein content and viscosity.....	+0.739	±0.021			
Series II						
Grade No. 4						
All 39	Viscosity and loaf volume.....	+0.561		4.122	>.01	0.410
35	Viscosity and loaf volume.....	+0.563		3.913	>.01	0.470
All 39	Protein content and loaf volume.....	+0.398		2.639	>.01	0.410
35	Protein content and loaf volume.....	+0.477		3.121	>.01	0.470
All 39	Protein content and viscosity.....	+0.767		7.190	>.01	0.410
35	Protein content and viscosity.....	+0.807		8.312	>.01	0.470
Series III						
Grades No. 5 and 6						
12	Viscosity and loaf volume.....	+0.563		2.151	=.05	0.708
12	Protein content and loaf volume.....	+0.656		2.747	=.02	0.708
12	Protein content and viscosity.....	+0.775		3.877	>.01	0.708
Series IV						
Garnet wheat, Grades No. 2 and 3 Northern						
24	Viscosity and loaf volume.....	+0.419		2.166	>.05	0.520
24	Protein content and loaf volume.....	+0.501		2.716	>.02	0.520
24	Protein content and viscosity.....	+0.947		13.828	>.01	0.520
1931 Crop						
217	Protein content and viscosity.....	+0.840	±0.020			

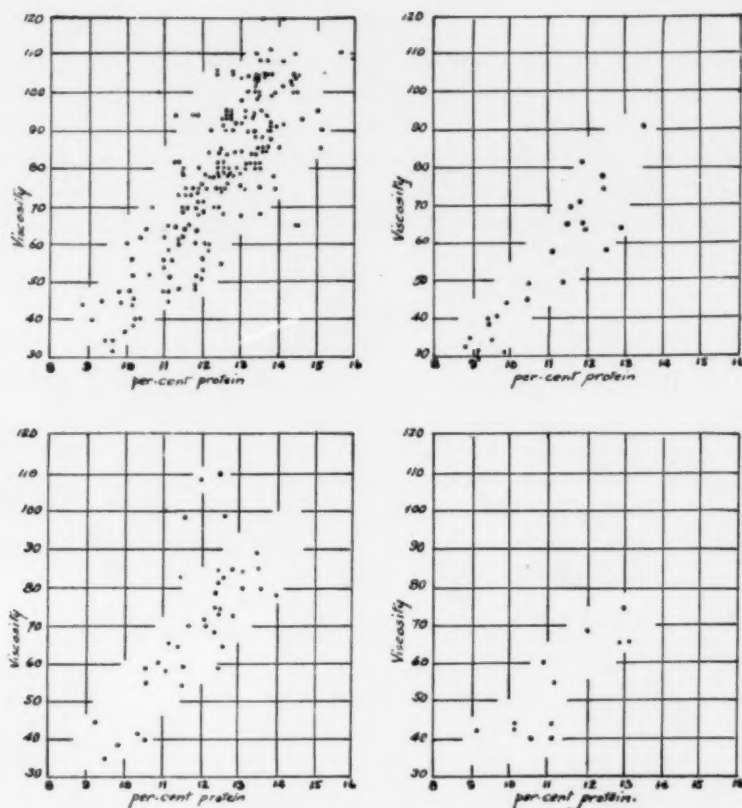


Fig. 1. Protein content and viscosity. Wheats graded No. 1, 2, and 3 Northern. 1930 crop, upper left.

Fig. 2. Protein content and viscosity. Wheats graded No. 4. 1930 crop, lower left.

Fig. 3. Protein content and viscosity. Wheats graded No. 5 and 6. 1930 crop, lower right.

Fig. 4. Protein content and viscosity. Wheats of the Garnet variety. 1930 crop, upper right.

parisons. Unfortunately, the samples were destroyed before the statistical examinations were made, so that a more detailed examination of these 4 samples could not be made. With these samples out of consideration, the statistical relationships were still higher. Other data given further on in this paper suggested that the ash content of these samples must have been abnormally high, and was responsible for their performance. The coefficients of correlation were not calculated for the relationship between baking scores and either viscosity readings or protein content as an inspection of the frequency table, Figure 2, showed that this relationship was very slight. The reason for this is obvious; the baking scores were greatly modified by color scores, and as color has not been proven to be a factor in determining strength, a baking score which includes color is of questionable value when dealing with strength.

Series III

Due to the fact that there was very little frost during the ripening period of the 1930 crop, this series consisted of only 9 samples of No. 5 wheat and 3 samples of wheat grading No. 6. A series of 12 samples is not large enough from which to draw accurate conclusions, but the coefficients of correlation and the values of t and the scatter diagram, Figure 3, indicate that the relationship between the variables is much the same as for the higher grades.

Series IV

The 24 samples of Garnet wheat, grading No. 2 and 3, were segregated in this series. A scatter diagram for this series is shown in Figure 4.

Larmour (1931) and others have demonstrated that Garnet wheat produces flour of lower quality than does Marquis wheat. Consequently, the 24 samples which were of this variety were studied separately. The coefficients of correlation for this series indicate the same relationships hold true as for the other series.

Viscosity Studies on a 1% Protein Basis

It was suggested that because the viscosity values were affected by the protein present in the flour, the viscosity values should be reduced to a uniform protein basis. Blish (1925) and Coleman, Dixon, and Fellows (1927) attempted to do this by dividing each viscosity value by the protein content of the sample involved. The coefficients of correlation obtained by this method for Series I are insignificant as can be seen from the following data:

Variable	Coefficient of correlation
Loaf volume and viscosity divided by protein	+0.178
Baking score and viscosity divided by protein	+0.280

This procedure, however, is inadmissible, because loaf volume is correlated with both viscosity and protein content; and the values, viscosity and protein content, are correlated with each other. The proper method of excluding the influence of protein content is to calculate the partial correlation coefficient between viscosity and loaf volume, holding the influence of protein content constant mathematically. The coefficients obtained by this method are also insignificant, as can be seen from the following:

Variables	Coefficient of correlation
Loaf volume—viscosity (protein constant)	+0.158
Baking score—viscosity (protein constant)	+0.244

Studies with the 1931 Crop

During the fall of 1931, 217 samples of wheat, grading No. 1, 2, and 3 Northern (and of the Marquis variety as far as could be judged), were studied. The coefficient of correlation between protein content and viscosity was $+0.840 \pm 0.020$. This is considered significant. The scatter diagram is shown as Figure 5.

Viscosity Studies on a Weighted Protein Basis

The results obtained with the 1930 crop samples suggested that viscosity was a function of protein content. If this is true, then samples containing the same amount of protein should have the same viscosity. Accordingly, 150 samples of the 1931 crop were tested by taking samples, each containing 2 gms. of protein, for a viscosity determination. By this method, of course, the weight of the sample varied according to the protein content. The results are shown in Figure 6. The samples all had about the same viscosity (within the same limits of error as the samples containing equal amounts of flour). In a later portion of this paper, it will be shown that small variations in ash content very probably accounts for the spread on the scatter diagrams.

The Regression of Viscosity on Protein Content

The regression lines of viscosity on protein content for the four series of tests with the 1930 crop and one series with the 1931 crop have been redrawn into Figure 7. The formulae for the lines are, approximately:

Series I, 1930 crop	$y = -130 + 16.7 (x).$
Series II, 1930 crop	$y = -110 + 14.1 (x).$
Series III, 1930 crop	$y = -88 + 11.8 (x).$
Series IV, 1930 crop	$y = -113 + 14.7 (x).$
Series I, 1931 crop	$y = -141 + 18.9 (x).$

Where y represents viscosity values and x represents protein content the regression coefficients indicate that there is a difference in hydration capacity between the proteins of sound and of damaged wheats, the difference increasing with the degree of damage and with increasing protein content. Series IV (Garnet series) had a coefficient of regression of 14.7 which is less than that for the corresponding grades of Marquis wheat, 16.7. The data for the 1931 crop are also interesting. The coefficient of regression was 18.9 which is higher than that for the 1930 crop.

These figures indicate that, in general, hydration capacity is lowered with the extent of damage of the wheat, and that the lower grades of wheat have lower quality proteins in this respect. Wheat grades, from these data, appear to be differentiated on the basis of hydration

capacity of their proteins. The data also suggest that hydration capacity differs from year to year, and that wheat varieties are also differentiated on the basis of the hydration capacity of their proteins.

Examination of the scatter diagrams for the variables, protein content and viscosity, shows that although the majority of the samples

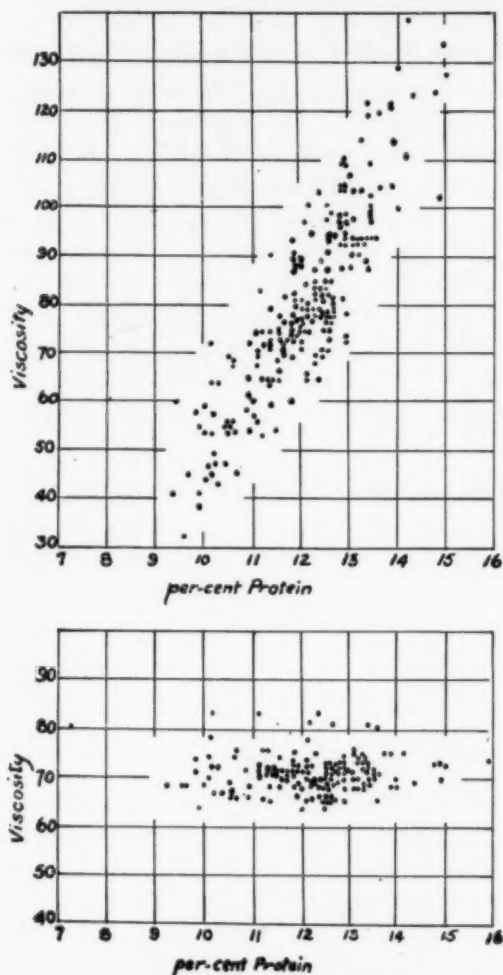


Fig. 5. Protein content and viscosity of a suspension containing 15 gms. flour, 1931 crop.

Fig. 6. Protein content and viscosity of a suspension containing 2 gms. protein, 1931 crop.

follow the regression lines closely, some samples are several units higher or lower than the mean viscosity for each particular protein content. There are several reasons for this spread. Experimental error will certainly account for some of it. Three determinations—moisture, protein, and viscosity—are involved; an error in any one of them affect-

ing the final data. However, the differences are too great in some cases to be explained by experimental error. It was suggested that those samples whose viscosity values were above or below the regression line

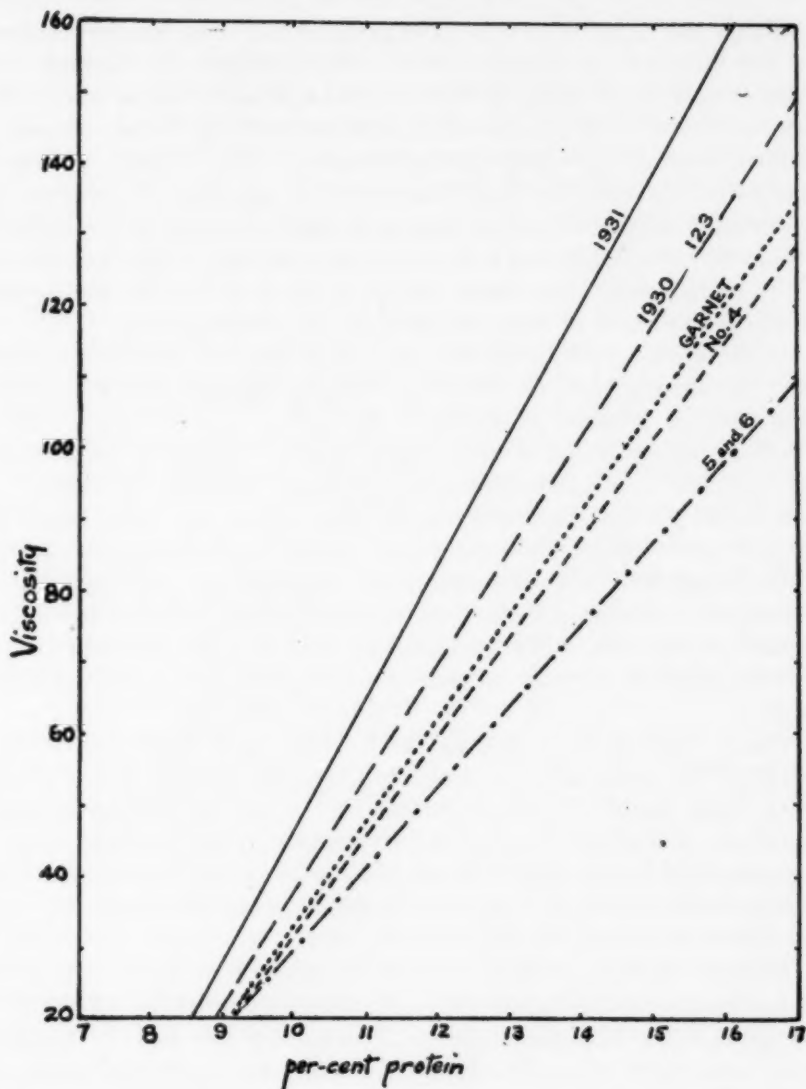


Fig. 7. Regression of viscosity on protein content. — 1931 Grades 1, 2, 3; --- 1930 Grades 1, 2, 3; ---- 1930 Grade 4; -.-.- 1930 Grade 5; 1930 Garnet.

were examples of high or low quality proteins. A statistical examination showed that those samples which were higher or lower than was to be expected from the regression lines showed no tendency to be

higher or lower in baking score or loaf volume than those samples which were close to the lines.

The Quality Factor "b"

Sharp and Gortner (1923) have pointed out that viscosity values are the resultant of several factors, among which ash content and quantity as well as quality of the proteins are of major importance. In their studies, soluble ash was eliminated by leaching the samples with distilled water before determining viscosity. The absolute viscosity values were corrected for the influence of the quantity of the proteins by determining the viscosities of several concentrations of suspensions and plotting the logarithms of the concentrations against the logarithms of the corresponding viscosity values. The slope of the line so obtained was taken to be a numerical value of gluten quality.

In the same investigation, Sharp and Gortner (*loc. cit.*) pointed out that, by plotting glutenin or protein content against viscosity in the same manner, a closer grouping of the curves was obtained. This, they believed, indicated a relationship between these two components and the imbibition phenomena. Also, as the result of leaching their samples before determining viscosity, the curves lay closer together and were more nearly at the same angle than was the case with the unleached samples. Six varieties were represented. Constant "b" was higher for flours milled from Turkey Red wheat than for flours milled from samples of Marquis wheat. Our data indicate that different varieties probably are different in the hydration capacity of their proteins.

These findings are concurred in by Blish and Sandstedt (1925) studying Nebraska wheats, and by Coleman, Dixon, and Fellows (1927), who found that the constant "b" offered no satisfactory explanation for the variation in baking strength of their samples.

Grewe and Bailey (1927), Vogel and Bailey (1927), and Hendel and Bailey (1924) also found no correlation between the constant "b" and baking strength of their series of flours.

In view of these findings, only 25 samples of the 1931 crop were studied by the quality factor "b." All samples graded No. 1 Northern and were of the Marquis variety. The soluble ash was not leached from the samples before determining viscosity as all samples contained $0.45 \pm 0.02\%$ of ash. Logarithmic viscosity-concentration curves were plotted. As was expected, all of the samples produced similar and parallel curves.

It is to be concluded from this superficial examination and from the relationship between protein content and viscosity, for experimentally milled flours of this study, that gluten quality even if measured by the

quality factor "b" is closely the same for all samples of one type of wheat. Data for a few samples of the 1930 crop indicate that the quality factor "b" was different for that crop. Unfortunately only a few samples were tested in this way so that the data are only suggestive as to differences in the quality factor "b" for different crop years.

These general findings as to quality are concurred in by Larmour (1931a), who used a baking formula containing 0.001% of potassium bromate. Larmour demonstrated that 82% of the variation in loaf volume of his experimentally milled flours could be accounted for by the *amount* of protein present, leaving only 18% of the variation due to errors in technique and to *quality* of protein. He considered that for his series of tests that quality of the protein was not an important factor. Apparently only sound wheats were used.

The conclusions to be drawn from this general study are in agreement with those of Geddes, Malloch, and Larmour (1932), who reported that ". . . within each grade of wheat, protein content is the most reliable index of strength . . . and that, in general, grades are clearly differentiated on the basis of gluten quality, as indicated by the decrease in the response to differential baking tests with lowering of the grade of wheat . . ." all of which signifies that within commercial wheat grades the proteins are of the same quality, as far as could be measured, although the quality of the proteins decrease as the grade of the wheat lowers. Newton and Cook (1930), studying the "bound water" of wheat flour proteins by cyroscopic methods, also arrived at the conclusion that all samples of flour have the same hydration capacity. They also apparently used, only sound flours.

Gortner (1924) stated that actual viscosity values are not necessarily related to the quality of the gluten present, on account of the differences in the amount of protein present in different flours. To eliminate this quantity factor, a factor representing the rate of change of viscosity per change of flour concentration was stated to be a quantitative measure of gluten quality. However, numerous investigators have demonstrated that this factor is not correlated with baking strength, as measured by baking tests. The data of the present paper indicate that the quality factor "b" is of little value.

PART II

Factors Influencing the Viscosity of Different Flour Grades

In the first part of this paper it was shown that the hydration capacity of the proteins of a series of 501 samples of experimentally milled flours of the 1930 and 1931 Saskatchewan crop, as expressed by the viscosity of flour-in-water suspensions, was very highly correlated with

the quantity of protein present when the samples were grouped on a basis of grade, variety, and year. In other words, the viscosity of suspensions of equal flour concentration varied strictly with the protein content of the samples.

When comparing commercially milled flours, or mill streams, the relationship is not so simple.

Smith (1925) concluded that, for short patents, viscosity values were in reasonable agreement with baking strength; that, for straight flours, the viscosity was lower; and for clear flours and low grades the results were inconclusive and contradictory.

Morgan (1924) obtained viscosity values for mill streams which were not directly a measure of baking quality, and which he was not able to interpret.

Durham (1925) in a study of mill streams of a Kansas mill also obtained viscosity values which were erratic and apparently not correlated with either protein content or baking strength.

Hendel and Bailey (1924) record similar findings.

Wood and Hardy (1908), Upson and Calvin (1916), Sharp and Gortner (1923), and others, have shown that the imbibition of wheat flours is markedly decreased by the addition of salts, and that the soluble salts present naturally in flours have an important effect on viscosity values. Sharp and Gortner attempted to eliminate, by preliminary leaching of their samples with distilled water, the influence of the ash content on the viscosity values.

Experimental

Material

In an effort to obtain more detailed information regarding the influence of the presence of salts or ash upon viscosity values, three series of mill streams were subjected to analysis. The data are given in Table II.

Methods

Viscosity Measurements: The viscosity readings were obtained by the method outlined in the early part of this paper with the following modifications. Preliminary tests showed that for experimentally milled flours, 5 cc. of N/1 lactic acid was sufficient to obtain maximum values. However, in the case of high ash flours, maximum values were not obtained until 8 or 9 cc. of acid were added. For this reason the viscosity values of the mill stream flours were obtained by adding 8 cc. of N/1 lactic acid to all samples of less than 0.65% ash, and 10 cc. to samples of higher ash content than 0.65%. It was later suggested that 5 cc. of 20% lactic acid would have been a more ideal addition. This is

TABLE II
ANALYSIS OF MILL STREAM FLOURS

Series A, 1930 crop				Series B, 1930 crop				Series A, 1931 crop			
Ash	Protein	Viscosity		Ash	Protein	Viscosity		Ash	Protein	Viscosity	
		15 gms. flour	2 gms. protein			15 gms. flour	2 gms. protein			15 gms. flour	2 gms. protein
P.ct.	P.ct.	Degrees	Degrees	P.ct.	P.ct.	Degrees	Degrees	P.ct.	P.ct.	Degrees	Degrees
0.34	11.20	91	94	0.35	12.20	88	88	0.33	11.70	117	106
.34	12.00	85	95	.38	12.30	78	71	.34	11.70	106	100
.35	11.40	76	76	.38	12.40	79	66	.34	12.20	124	104
.35	11.50	93	71	.39	12.10	68	74	.35	11.40	119	114
.35	12.10	90	82	.43	12.00	64	73	.35	11.90	112	108
.40	11.30	78	72	.43	12.70	62	57	.38	11.50	99	90
.40	11.30	78	72	.44	12.10	60	70	.38	11.80	106	100
.40	11.70	72	68	.47	13.70	68	49	.38	14.20	131	85
.41	11.50	71	65	.49	16.90	123	53	.42	12.60	86	77
.41	11.70	67	62	.52	11.90	40	47	.42	16.00	162	77
.41	13.80	102	62	.55	12.70	46	40	.43	11.50	77	76
.41	15.30	134	61	.57	14.20	57	42	.44	11.90	82	75
.44	12.50	70	72	.60	16.10	73	37	.44	13.30	94	80
.45	11.30	58	56	.61	12.60	41	34	.45	15.70	136	74
.46	14.50	91	50	.61	13.20	34	30	.46	11.90	62	64
.49	11.60	55	54	.62	12.70	48	36	.46	11.90	63	77
.49	12.90	63	54	.65	15.80	60	30	.46	15.30	110	72
.51	14.60	85	46	.69	13.40	33	28	.53	14.90	96	56
.52	12.00	49	40	.73	21.20	120	20	.54	13.00	64	57
.52	13.90	70	40	.75	14.80	38	24	.54	14.70	90	57
.53	13.70	44	67	.77	13.70	26	23	.56	13.30	68	47
.54	14.40	71	39	.78	13.10	24	22	.57	14.00	72	54
.58	13.00	58	36	.80	14.30	32	23	.59	14.20	69	52
.59	12.70	47	37	.97	17.70	31	16	.60	14.20	70	43
.65	15.00	40	23	1.16	23.10	54	14	.62	11.90	44	42
.66	13.80	46	30	1.20	14.80	19	10	.75	13.70	50	27
.66	14.30	52	33	1.32	15.20	5	3	.76	13.50	35	29
.70	13.00	44	29	1.65	15.50	10	4	.76	14.70	50	27
.71	13.10	40	24	1.65	19.50	7	2	.76	15.30	41	32
.76	18.80	72	35	1.78	19.80	5	2	.82	15.70	37	21
.77	14.80	48	30	2.10	17.70	14	5	.84	20.00	73	30
.82	14.90	34	18	2.30	19.60	10	3	.86	14.80	27	19
.97	17.30	15	4					.87	16.30	30	22
								1.08	13.30	33	13

equivalent to about 11 cc. of N/1 lactic acid solution. Two sets of viscosity readings were taken; one of 15 gms. (moisture free basis) of flour, and one of samples containing 2 gms. of protein.

As previous investigators have pointed out, the viscosity values, at least when dealing with flours of different grades, were apparently not related to protein content, and (data not included) the coefficient of correlation between viscosity and baking strength was low and erratic, particularly for the samples containing more than 0.45% ash.

As the viscosity of lyophylic systems is greatly altered by the presence of electrolytes, Sharp and Gortner (1923)* attempted to eliminate this depressing effect by a preliminary leaching with distilled water. It was assumed that by doing this practically all of the soluble

ash was extracted and decanted from the sample, and that the viscosity values then obtained were a measure of the inherent hydration capacity of the proteins in each individual sample.

Correlation between Viscosity and Protein Content of Mill Stream

The data given in Table II were subjected to a statistical analysis, results of which are given in Tables III and IV. As other investigators

TABLE III
SIMPLE CORRELATION COEFFICIENTS OBTAINED WITH MILL STREAM FLOURS

Factors involved	Description of samples		
	Series A, 1930 crop	Series B, 1930 crop	Series A, 1931 crop
N	31	24	30
Protein \times viscosity	-0.186 ± 0.173	$+0.344 \pm 0.137$	-0.019 ± 0.182
Protein \times ash	$+0.701 \pm 0.091$	$+0.479 \pm 0.158$	$+0.380 \pm 0.156$
Viscosity \times ash	-0.820 ± 0.058	-0.658 ± 0.117	-0.809 ± 0.063

TABLE IV
PARTIAL CORRELATION COEFFICIENTS OBTAINED WITH MILL STREAM FLOURS

Factors involved	Description of samples		
	Series A, 1930 crop	Series B, 1930 crop	Series A, 1931 crop
N	31	24	30
Protein \times viscosity (ash constant)	$+0.970 \pm 0.009$	$+0.997 \pm 0.008$	$+0.973 \pm 0.009$

have reported, the correlation between protein content and viscosity was very low, while the viscosity seems to be more highly correlated with ash content. In either case, simple correlations are misleading, because both ash and protein content have important and opposite effects on viscosity. Therefore, for the three series, partial coefficients of correlation were calculated for the relation between viscosity and protein content. By this computation the effect of ash content was eliminated, and the results are such as would have been obtained if the ash content had been constant. Partial coefficients of correlation between protein content and viscosity were found to be very high (more than 0.900 in each case), and substantiated our previous statement that the inherent hydration capacity is related almost altogether to the amount of protein present.

Sharp and Gortner (1923) noted that two effects were obtained by leaching the soluble ash from their samples before determining viscosity. First, the viscosity readings were always higher; and second, that

the differences between samples became less, i.e., that all samples approached the same viscosity. In order to determine why differences in viscosity were still obtained after leaching, the following experiments were carried out:

The Extent of the Depression of Ash Content on Viscosity

It has been assumed that the depression caused by ash content is a linear function. If this is true, then leaching, which extracts *most* of the soluble ash from the suspension, will eliminate *most* of the depression on viscosity. That this relation is *not* linear is shown very clearly in Figures 8 and 9. In order to eliminate the effect of protein on

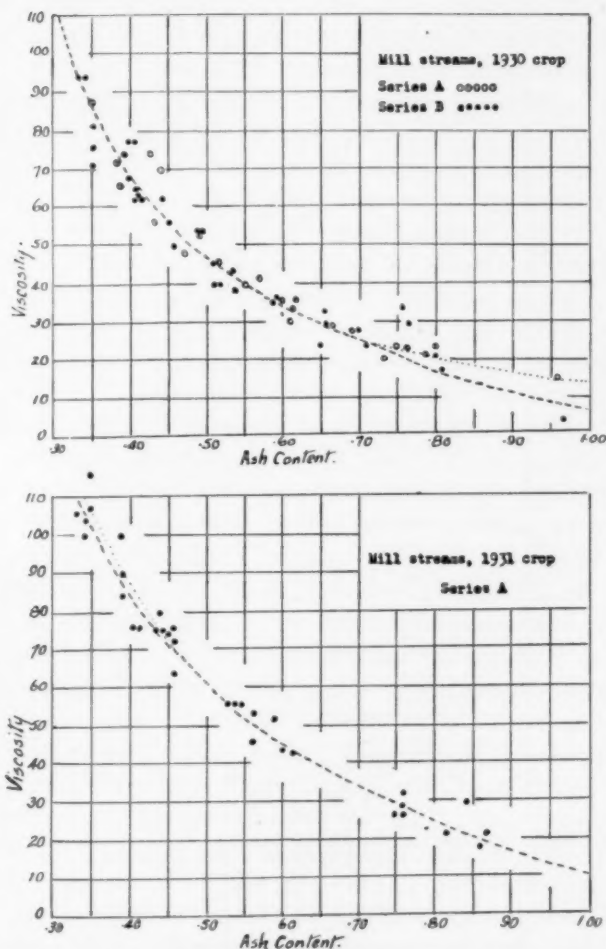


Fig. 8. Ash content and viscosity of mill streams. 1930 Saskatchewan crop. Viscosity determined on samples weighed to contain 2 gms. of protein.

Fig. 9. Ash content and viscosity of mill streams. 1931 Saskatchewan crop. Viscosity determined on samples weighed to contain 2 gms. of protein.

viscosity, samples were weighed to contain 2 gms. of protein and the viscosity determined. Results are shown in Table II, last column; and in Figures 8 and 9. The ash content of the samples were plotted against the viscosity of suspensions containing equal amounts of protein. It can be seen that the relation is not linear, in fact it is logarithmic from 0.35% to about 0.75% ash, the region in which all except the low grade flours are found. That the relation is a logarithmic one is further shown in Figure 10, where the logarithms of the ash contents

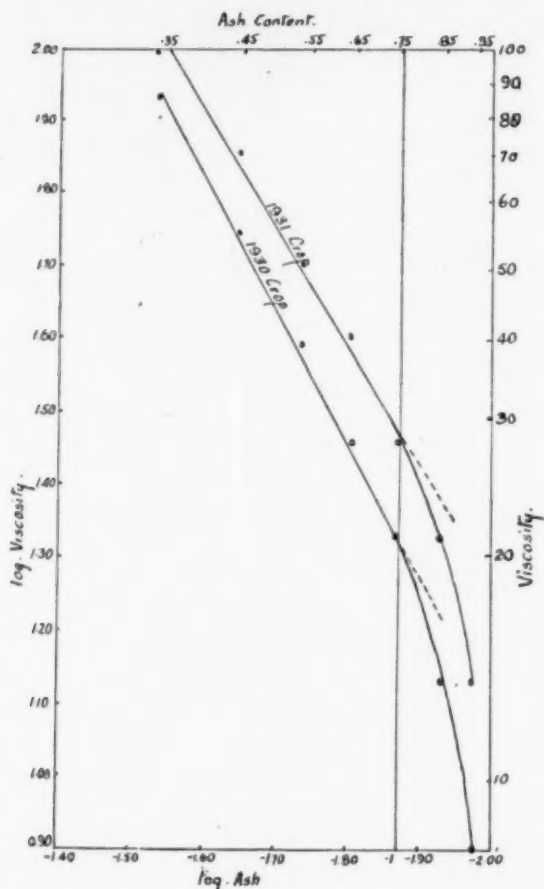


Fig. 10. Logarithms of ash content and logarithms of viscosity of mill streams. Viscosity determined on samples weighed to contain 2 gms. of protein.

are plotted as ordinates, and the logarithms of the viscosity values as abscissa. A straight line was obtained between 0.35% and 0.75% ash content, beyond which the line slopes downward as in Figures 8 and 9, indicating that the curve deviates slightly from the logarithmic.

The equation for the logarithmic curves is of the form, $\log. \text{viscosity} = a + b (\log. \text{ash content})$, and the lines have the approximate equations:

$$1930 \text{ crop, } \log. \text{viscosity} = 4.66 + 1.76 (\log. \text{ash content}).$$

$$1931 \text{ crop, } \log. \text{viscosity} = 4.64 + 1.68 (\log. \text{ash content}).$$

In the magnitude of about 0.55% or 0.60% ash, the extent of the depression caused by ash content is not very great, while in the region of 0.30% or 0.35% ash content the depression is very high per increase of 0.01% ash. After leaching with distilled water, the suspensions contain only very small amounts of ash and would therefore be in the region represented by the left hand side of the curves in Figures 8 and 9, where the depression of viscosity per increase of very small amounts of ash is very great. Unless the manipulation of leaching is carried out with extreme care and completeness, small differences in ash content of the suspensions after decantation would be almost certain to occur with corresponding differences in viscosity caused not by differences in the inherent quality of the proteins but by differences in the ash content of the suspensions. It would appear, therefore, that a better method of bringing all samples to the same ash content would be to add a salt to the suspensions in order to bring all samples to the same electrolyte content in the neighborhood of, say, 0.75% ash, where the depression caused by small differences would not be so great. In any case, whether the ash content was zero, 0.20%, or 0.75%, our data indicate that the viscosities would be a function of protein content or probably, as Sharp and Gortner (1923) point out, to be more correct, of glutenin content, with the variations caused by different types of wheat such as were indicated in an earlier part of this paper.

That the depression caused by ash content is curvilinear is further demonstrated by the following experiment: Finely ground bran was sifted on a 14 XX silk sieve. The 'throughs' were in the form of a very fine, light brown powder containing 3.28% ash, and 21.30% protein (which was assumed to be non-gluten proteins and, therefore, had no effect on viscosity). Varying amounts of this bran powder were thoroughly mixed with a high patent flour of 0.35% ash and 11.60% protein content. Assuming that the gluten protein content was not changed, the samples were weighed to contain 2 gms. of protein, based on the protein content of the original flour, and the viscosity determined on 5 samples. The viscosity values almost exactly coincide with the curve in Figure 8.

It was suggested that bran particles might perhaps tend to break down or "loosen" the colloidal structure, which would account for the decrease in viscosity. Accordingly, a concentrated water extract

of bran was prepared, and added to the flour suspension in increments of 0.5 cc. The results were similar to those obtained with powdered bran, and the data need not be repeated here.

TABLE V
VISCOSITY OF A HIGH PATENT FLOUR (1930 CROP) AFTER ADDING VARIOUS AMOUNTS OF BRAN POWDER

Ash content	Protein content	Gluten content	Viscosity
P.ct.	P.ct.	P.ct.	Degrees
0.35	11.60	11.60	86
0.47	12.10	11.60	60
0.53	12.30	11.60	42
0.61	12.50	11.60	32
0.75	12.90	11.60	19

Similar experiments were conducted, using solutions of various salts. The solutions were added in increments of 0.5 cc. and curves similar to those in Figures 8 and 9 were obtained. From this preliminary examination, there seemed to be a lyotropic series, as the curves obtained from different salts did not coincide. It may be noted here that Erlenbaugh (1926) obtained similar results by adding mono-calcium phosphate to flour.

Discussion

The data obtained from a study of three sets of mill stream flours substantiate the previous statement that the inherent hydration capacity of the proteins of wheats of the same variety, crop year, and degree of damage (if any), is the same for all samples. There may be differences in the inherent hydration capacity of the proteins of different types of wheat. The mean correlation coefficient (keeping the effect of ash a constant) between viscosity and protein content for three sets of mill stream flours was $+0.980$.

As other investigators have pointed out, viscosity values are seemingly meaningless when dealing with flours of different grades. However, it has been shown that viscosity is determined by two factors beside hydration capacity—ash and protein content. When the effect of ash is partially eliminated by preliminary leaching with water, erratic results still occur due to the large effect of small differences in ash content when the suspensions are in the region of low ash content. This has been demonstrated by eliminating the effect of protein content by determining the viscosity of samples containing equal amounts of protein. The influence of ash was shown to be curvilinear, the greatest depression being in the region of low ash content. When the effect of ash content was eliminated by the method of partial correla-

tions, it was clearly shown that there is no difference in the inherent imbibitional strength of the proteins between any of a set of mill stream flours.

Examination of Figures 8 and 9 indicates that the depression of viscosity caused by ash content was greater for the 1930 crop than it was for the 1931 crop.

It was also shown in the first part of this paper that the hydration capacity of wheat proteins was lower for the 1930 crop. This corroborates the statement that, although there is no difference in the inherent colloidal structure of the proteins (as regards hydration capacity) for for one type of wheat, there may be differences from year to year. If it had been possible to investigate it, differences in the depression of viscosity due to ash content between varieties and commercial wheat grades would, in all likelihood, also have been demonstrated.

It will be noticed that in computing coefficients of correlation, several of the very high ash stream flours were not included. The reason for this was that suspensions of these samples, when acidulated with lactic acid, were not viscous, but acted similar to a starch suspension. The viscosity of the protein sol, after being depressed by the large amount of ash, was very low, and actually was nearly zero. These samples also had very high protein contents. Apparently, either the plasticity of the starch suspensions masked the viscosity of the protein sol and the sample acted as a plastic suspension rather than a viscous sol, or the total protein in the high ash streams from the "tail of the mill" could not be taken as representing gluten content, and the correlation between total protein and viscosity would not be the same as the correlation between viscosity and glutenin, which is apparently (from the work of Sharp and Gortner (1923)) the protein responsible for the phenomena of viscosity. These streams cannot be properly considered as flours, in a strict sense, and, therefore, do not enter into our discussion anyway without further study as to the nature of their proteins.

Gortner and Doherty (1918) in summarizing their data obtained from a study of the imbibition of the proteins of a series of five flours, stated that although the moist gluten from the flours differed widely in their quality and physical properties, they were hydrated to almost the same extent, but that the gluten from the weak flours became dispersed and lost their tenacity before maximum imbibition occurred, while the strong glutes imbibed water to their maximum capacity and still remained coherent.

Upson and Calvin (1916) concluded that the quality of gluten is regulated by the kind and concentration of the salts and acids present in the dough. If they are such as favor imbibition, the quality will be

poor, whereas the presence of acids or salts such as tend to inhibit absorption makes for an improved gluten.

Earlier, Wood and Hardy (1908) had come to the conclusion that variations in coherence, elasticity, and water content are due rather to varying concentration of acids and salts in the natural surroundings of the gluten than to any intrinsic differences in the composition of the gluteins themselves.

Kress (1924) believed that, from his experience, gluten quality does not influence viscosity as much as ash content does.

Gortner and Doherty (1918) could not reconcile the conclusions of Wood and Hardy, and of Upson and Calvin, with their observations. They recognized that inorganic salts, when added to an acid solution, lower the imbibition of gluten placed in such solutions, but maintained that acid and salt concentration are not responsible for the differences between a strong and a weak gluten, although their own statement, given above, makes it clear that hydration capacity cannot be responsible for the differences.

Newton and Cook (1930), who studied the problem by cyroscopic methods, showed (from their experiments) no differences in the hydration capacity of strong and weak flours. Their conclusions were that gluten quality must be attributed to other properties than hydration rates or capacities of the gluteins.

The data presented in this study partly reconcile these two points of view. It has been shown that, for one type of wheat the inherent hydration capacity of the proteins is practically the same for all samples, or if taken on the basis of the amount of flour, is very highly correlated with the total protein content, as a linear function. With flours of different grades, imbibition capacity is dependent upon the concentration of ash as well as protein.

In comparing wheats of different crop years, varieties, and degree of damage by frost, etc., the data indicate that differences in the inherent maximum hydration capacity of the proteins are present. It seems peculiar that wheats grown in different crop years should show differences in hydration capacity, when wheats grown the same year in various localities and under the most diverse weather and soil conditions should be the same in this respect. Nevertheless, there was a decided difference between the 1930 and 1931 crops. Observations in general seem to bear out the fact that there may be differences in quality from year to year.

Wheats possessing different degrees of damage have proteins of different qualities. Our data indicate that one of the differences lies in their hydration capacity.

That Garnet wheat possesses proteins of lower quality than Marquis

has been known for some time to the milling industry. Larmour (1931a) has shown that Garnet is lower in quality than Marquis. Our data, that the hydration capacity of the proteins of Garnet wheat is lower than those of Marquis, are a further indication that the proteins of Garnet are lower in quality.

In view of our other conclusions regarding the viscosity of flour-in-water suspensions, it does not seem reasonable that the quality factor "b" method of measuring gluten quality should give any information regarding the inherent colloidal structure of the proteins. In examining experimentally milled flours of approximately the same ash content it was found that the factor "b" was the same for all samples. Due to the fact that very small errors in viscosity readings cause very great errors in the logarithmic curves, this method is less accurate than taking the actual viscosity values themselves. The samples of Series A, 1931 crop, were also tested by this method. The coefficient of correlation between ash content and the factor "b" was found to be -0.754 ± 0.090 , which indicates that the factor is highly correlated with the ash content of the flour. Erratic results would be obtained if the samples were first leached with distilled water, as then the ash content would greatly influence the viscosity values.

Conclusions

Data in the present study may be summarized as follows:

Viscosity values, obtained by the method described, and total protein content are about equal in value in predicting the baking strength of experimentally milled flours.

For flours of the same wheat variety, and with the same degree of damage, such as occurs during ripening, and of the same crop year, viscosity values or, in other words, hydration capacity of the proteins is very highly correlated with total protein content.

The relation between viscosity and protein content is linear between 9% and 16% protein, the limits of our study.

There is a difference in hydration capacity between the proteins of wheats possessing different degrees of damage. This has been demonstrated by the decrease in the coefficient of regression of viscosity on protein content with the lowering of the commercial grade of the wheat.

There was a difference in the hydration capacity of proteins from wheats of the two years studied.

The proteins of the Garnet variety have a lower hydration capacity than those of Marquis wheat of the same grades.

If hydration capacity, as measured by the torsional viscosimeter, is a measure of protein quality, then there is only a slight difference be-

tween the proteins of wheats of one commercial grade, variety, and crop year. The commercial grading system apparently differentiates between wheats on a basis of the hydration capacities of their proteins.

The quality factor "b," as measured by the method of Sharp and Gortner (1923), gives no information with experimentally milled flours. With flours of different ash contents, this factor is apparently determined by the ash content of the flour.

With flours of different extractions, the relation between observed viscosity values and other factors is complicated by the great influence of ash content on the viscosity of the suspensions.

The relation between ash content and viscosity is curvilinear, the influence of ash content on viscosity values increasing logarithmically towards the region of low ash content.

The influence of ash content varied with the two years studied, and probably (from the data of the experimentally milled flours) would with different varieties and with different wheat grades.

When the influence of ash content is eliminated by statistical methods, it is evident that the inherent hydration capacity of the proteins of any sample of flour of the same crop year (and probably variety and wheat grade) is practically a constant.

The method of eliminating the effect of ash content by leaching with distilled water is inaccurate.

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A STUDY OF PROTEINS OF WHEAT BRAN

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Introduction

The greater part of the immense study which has been made of proteins of wheat have been upon the flour. Few papers have been devoted especially to the proteins of the bran and yet the nitrogen compounds in the bran contain more than one-fourth of the total combined nitrogen received when wheat is bought on protein content. A knowledge of these compounds is of importance when studying the grain for the kind of flour it will make. It is also important as an aid to understanding the nature of the proteins in the grain and in the flour, especially those grades of flour which contain appreciable quantities of the bran. Such knowledge will also help us to avoid erroneous conclusions on some of the subjects in which bran and wheat have a part.

Osborne and Voorhees (1893), and later Osborn (1907), published data with respect to the proteins of the wheat kernel. In these papers no clear distinction was made between the proteins of the flour and the proteins of the bran. In several instances, the proteins were separated from shorts, which consists largely of bran, and the composition of proteins from them was found to be substantially the same as for those of the same type found in the flour. Jones and Gersdorff published two papers (1923, 1925) dealing especially with proteins of bran. They separated from a bran, an albumin, a globulin and a prolamine. In their analyses of these proteins, as they separated them, they found distinctly less nitrogen than was found by Osborne in his analysis of the same types of compounds. Teller (1896), in a series of investigations on the proteins of wheat and mill products, found distinctly less gliadin and relatively more albumin and globulin in commercial bran than in the flour from the same wheat, and found little gliadin in the outside seed coating (epicarp) which was separated in a special dust room in the mill from which the samples were obtained.

In this paper, we are giving the results of a similar but more extended separation of the nitrogen compounds in 12 samples of very pure bran prepared from brans obtained from several representative mills in different parts of the United States. These include bran

milled from different classes of wheat. We are also giving for comparison, results obtained by the same methods of analysis on a sample of bran and endosperm milled from wheat, in our laboratories, in such a manner as to give bran as pure as the other bran samples, and endosperm of high purity.

Forms and Characteristics of Nitrogen Compounds in Wheat

The chief forms of nitrogen compounds of wheat are:

(1) Albumins; (2) globulins; (3) prolamines; (4) glutelins; (5) those proteins which are not dissolved from the finely divided bran by cold dilute alkali; and (6), an appreciable quantity of non-protein nitrogen compounds. The properties of these several forms of nitrogen compounds, so far as they are pertinent to this discussion, are substantially as follows:

(1) Albumins are a class of protein compounds common to plants and animals. They are soluble in water and dilute salt solutions, but insoluble in alcohol. They form soluble acid and alkali albuminates when brought in contact with acids or alkalis. They are coagulated by heat below the temperature of boiling water and may be removed from dilute salt solutions in this way.

(2) Globulins are common to plants and animals. They are found in various seeds from which they may be separated and obtained in the form of crystals. They are apparently less abundant in wheat than the other leading proteins. They are soluble in dilute salt solutions from which they may be precipitated by the addition of alcohol.

(3) Prolamines are a type of protein which apparently belong distinctly to cereals. The chief prolamine of wheat is gliadin. Whether there are other forms of prolamines present differing somewhat from gliadin is not material at this time. If present, the properties are not sufficiently different from gliadin to interfere with their treatment as such. Gliadin is freely soluble in dilute alcohol of varying strengths. As it occurs in flour and wheat meal it is practically insoluble in 95% alcohol. After this treatment it is not dissolved by further treatment of the product with more dilute alcohol. Apparently it is in part changed in a similar manner by less concentrated alcohol. For this or other reasons, it is less dissolved from fine wheat meal and flour by 70% or 75% alcohol than by 60% alcohol, or even that which is more dilute. The solubility in dilute alcohol as in water, varies with conditions especially as to acidity. Gliadin can be recovered from solutions apparently unchanged by evaporating at a low temperature, the exact limit of which we have not determined (45° C. is not too high). If the solution is evaporated at a high temperature near the boiling point of water, it is in part coagulated and becomes insoluble in water and in

alcohol. Gliadin is dissolved from wheat meal by water and dilute salt solutions. The amount of solubility decreases with the concentration of the salt solutions, but is appreciable even in 10% sodium chloride and stronger. Like quantities of different wheat products digested at room temperature, with the same strength of salt solutions, yield practically the same amount of gliadin to the solution, as was early demonstrated by Teller (1896). At that time it was found that when 5 gms. of wheat flour, fine wheat meal, or fine bran were digested for $2\frac{1}{2}$ hours with 250 cc. of a 1% solution of common salt, the average amount of gliadin which entered into solution in 50 cc. of the liquid was equivalent to 0.27% nitrogen calculated on the 1 gm. of sample which it represented. Similar results as to solubility have since been obtained with this or other salt solutions by a considerable number of workers, among whom may be mentioned: Snyder (1899), Hoagland (1911), Olson (1914), and Sharp and Gortner (1923). To overlook this point, as in the present A. O. A. C. official method for determining albumin and globulin, introduces a gross error. It is also a point of importance in attempting to recover the gliadin from wheat products, especially bran, after previous extraction with water and salt solutions.

(4) Glutelins are a type of protein also distinctive of cereals, and some have claimed that glutenin is present in wheat only. These compounds are not more than slightly soluble in water, in dilute salt solutions, and in alcohol. They are readily soluble in dilute alkali from which they may again be precipitated by carefully neutralizing with acid. Excess of acid will re-dissolve them. The nitrogen present is close to the amount found in gliadin. Like gliadin, the separation ofutelins from bran in a pure form is extremely difficult because of the presence of other compounds of similar solubility but containing no nitrogen.

(5) When finely divided bran is extracted with dilute alkali at room temperature, an appreciable proportion of nitrogenous substances remain undissolved. The amount undissolved decreases as the bran is made more fine. Whether it is a different type of protein, or whether there are other causes which prevent its entering into solution, have not been fully determined. Long, continued extraction does not materially increase the amount of protein dissolved by 0.2% sodium hydroxide over what may be obtained in an automatic shaker for 3 hours.

(6) The non-protein nitrogen compounds are soluble in water, dilute salt solutions, and in alcohol. They are not thrown out of water and dilute salt solutions by certain reagents which remove the dissolved proteins. A discussion of this class of compounds in cereals has already been published (Teller, 1932). The non-protein compounds in bran

do not consist entirely of asparagin or glutamine. This was found out when the non-protein compounds extracted by water from 4 gms. of bran were boiled with dilute hydrochloric acid for 1 hour; the acid nearly neutralized with sodium hydroxide and an excess of magnesia added. The nitrogen recovered in the ammonia distilled off was only one-eighth of the total non-protein nitrogen in solution, indicating that one-fourth of this non-protein nitrogen was from asparagin. No free ammonia was found by distilling with magnesia a portion of the solution which had not been digested with acid.

Before entering into a detailed study of the several brans, the results of which we are giving below, an effort was made to separate the several proteins from one of them in pure enough form for analysis. After a considerable amount of very careful work in this attempt, it was very apparent that the large amount of soluble matters other than protein, prevents the successful separation and purification of the proteins from bran to such an extent that the results are far from satisfactory. While albumins were recovered which showed nitrogen close to the commonly accepted amount, and a small quantity of gliadin was recovered which showed more than 15% nitrogen, these products were clearly impure. When an extract of finely divided bran was made with 70% alcohol, the product obtained, by the usual methods of separation and purification, contained such large quantities of materials other than protein that the amount of nitrogen in the product was less than 12%. This was true after repeated re-solutions and reprecipitations with stronger alcohol in the commonly accepted manner and after all fat had been removed. After extracting a portion of the bran with water, salt solutions, and dilute alcohol, protein was recovered from it by dissolving in 0.2% sodium hydroxide. This protein was precipitated on the addition of acid, but other materials which responded to these reagents in a similar manner could not be separated from the protein, and when the preparation was recovered it was found to be far from pure. It should be remembered that the Kansas bran used for this purpose was very pure, well-freed from flour, and contained no appreciable protein from other parts of the grain.

The difficulty of separating the protein of cereals in a pure form has long been recognized and early led to the determination of nitrogen and multiplying this nitrogen by a factor suitable to show the amount of protein. The methods of isolating and identifying the several proteins of cereals are quite well established and have been very minutely described by Osborne and his associates. It would not be practicable to compare a large number of products by the methods which they used. They isolated the different proteins as completely as possible and

weighed them. The process was often long and laborious and there was unavoidable loss in the purification.

That it is possible to make a quantitative determination of the several nitrogen compounds without isolating them was long ago demonstrated (Teller, 1893). Separations were made in such a manner that the nitrogen belonging to each could be determined. These methods met with the approval of Osborne so well that he caused them to be shown in detail in a leading work on food analysis by Leach, revised by Winton (1920).

In these methods as followed by Teller (1896 and 1898), a 1% salt solution was selected for various extractions. In the work on brans, which we are here describing, it was found that a 5% salt solution gave somewhat higher results, and it has accordingly been used for this work. We also used a 60% alcohol in preference to a stronger solution for similar reasons. We have also included in these determinations the amount of matter insoluble in dilute alkali as giving added information. The method of separating the albumins and globulins by boiling was described in the earlier work but was not carried out in detail. This procedure is of exceptional importance in an examination of the proteins of bran. Osborne (1907, p. 115) believed the source of the globulin to be chiefly in the embryo. It is apparent, from the results here given, that it is also contained to a very considerable extent in bran and to a lesser extent in the pure endosperm.

Experimental

The methods used in these examinations were as follows:

Methods

Total nitrogen: Total nitrogen was determined on 1 gm. of substance by the modified Kjeldahl method, using mercury and sodium sulphate.

Albumin nitrogen was determined by digesting 10 gms. of the fine bran with 500 cc. of a 5% solution of pure sodium chloride at room temperature with frequent shaking for a period of 4 hours. The solution was then filtered clear. 100 cc. of this solution were transferred to a Kjeldahl flask and heated to a temperature of 95° C. for one-half hour. On the following morning the precipitate was collected on an 11 cm. S. S. filter paper No. 589, white ribbon, washed once with 10 cc. of water after which the filter with its contents was returned to the flask and the nitrogen determined as before. The nitrogen obtained was from 2 gms. of substance.

Globulins: For the determination of globulin 200 cc. of U. S. No. 30 alcohol were added to 50 cc. of the above described clear salt solution which had been placed in a Kjeldahl flask. The precipitate which

formed was allowed to settle until the next day when it was collected on a filter and the nitrogen in it determined as for the albumin. This nitrogen represents 1 gm. of substance. The difference between the alcohol precipitate nitrogen and the nitrogen from the precipitate at 95° C. gives the nitrogen in the globulins.

Non-protein nitrogen: The non-protein nitrogen was determined by precipitating the proteins from 100 cc. of the above described clear salt solution by adding 25 cc. of a 10% solution of phosphotungstic acid in 3% hydrochloric acid. The nitrogen was determined in 100 cc. of the clear filtrate obtained after standing for 2 hours or more. The amount of nitrogen thus obtained represents the non-protein nitrogen from 1.6 gms. of the sample.

Prolamines: Prolamine nitrogen was obtained by digesting 2½ gms. of the sample in 125 cc. of 60% neutral alcohol for a period of 40 hours, at a temperature of 45° C., with frequent shaking. This length of time for extraction is not necessary but was followed. The alcohol was thoroughly removed from 50 cc. of the filtrate obtained from this extraction by heating on a sand bath after adding ½ cc. of strong sulphuric acid. The nitrogen was then determined in the residue in the usual manner and represents the amount of alcohol soluble from 1 gm. of the bran. Alcohol under these conditions extracts the prolamine and non-protein compounds. The nitrogen of the former is obtained by subtracting the non-protein nitrogen from the total alcohol soluble nitrogen.

Nitrogen compounds soluble in dilute alkali: These were determined by digesting 4 gms. of the bran with 200 cc. of a water solution containing 0.2% sodium hydroxide. The digestions were carried on by frequent shaking, at room temperature, for a period of 40 hours, although 24 hours was found to be sufficient to give substantially the same results as by shaking in an automatic shaker for 3 hours. The nitrogen was determined in 50 cc. of the filtrate from the extraction and is from 1 gm. of the bran. The insoluble in dilute alkali was obtained by subtracting the soluble nitrogen from the total. The glutelin nitrogen was obtained by subtracting from that soluble in alkali, the sum of the nitrogen of albumin, globulin, prolamine and non-protein compounds. It is hardly necessary to state that due care as to details, as recognized by competent chemists, is necessary in all these determinations.

These methods are based largely upon the methods used by Osborne and others for isolating the several proteins of wheat to determine their composition and properties. The work of Osborne and his associates was extensive and of great thoroughness. The more recent studies of others, while finding some apparent discrepancies, have not

overturned it. In recent discussions some confusion has arisen because of incompleteness of work done, as well as because of the unknown extent to which the proteins are changed by various agents used and the unusual properties of some of these proteins, especially the prolamines. An adequate discussion of this would require an extended critical review which would be out of place in this article

It is recognized that in nearly all chemical separations some imperfections are likely to occur. This is increased with organic substances, especially those of a protein nature. A long continued and careful study of the subject matter of this article has strengthened the early beliefs of one of us that the methods here used and the deductions drawn from the results obtained are correct in the main and give a substantial foundation for further investigation and study.

Material Used

Data from a single product are hardly sufficient as a basis for drawing general conclusions of great importance. We have felt that a considerable number of brans should be compared to find what difference may appear in brans milled from different classes of wheat. A 2 pound sample of a clean bran was obtained from each of 12 mills. These were chosen as likely to give a representative product from wheats produced in their several sections. The hard spring wheat brans used were milled from Minnesota wheat, mixed northern Minnesota and North Dakota wheat, and from wheat grown in southwestern Montana. The hard winter wheat brans were from central Nebraska, southern Kansas, central Oklahoma, and northern Texas. The soft winter wheat brans were from Missouri, southern Illinois, southern Michigan, and a Washington white wheat. One durum wheat bran was also included. Besides these, we have milled in our own laboratory, a Marquis seed wheat and a white winter wheat from southern Michigan. The latter type of wheats for this season are reported to be unusually high in protein.

These several brans, as received, were well bolted over gritz gauze, rejecting that which passed through a No. 70. They were then further reduced on fine corrugated rolls until all passed through a No. 40 sieve. Only that bran was retained which would not pass through a No. 70 sieve. In this manner, a goodly quantity of each bran was obtained in a fine state of granulation and well-freed from adhering flour. Naturally a very little flour still remained, especially on bran from the soft wheats. In making the liquid extraction, very small quantities of starch was found at the bottom of the liquid. The amount of starch in one of the spring wheat brans as thus prepared, was found to be a little more than 2%. The brans from the softest wheats contained

a somewhat greater amount. The so called aleurone layers of cuboidal cells on the innerside of the bran were still adherent. Naturally, considerably fine bran passed through the finer sieve but there is no reason to believe that it is of different composition than that which was used for the analyses. A microscopic examination of it showed much of it was cut fine with the several layers of the bran intact.

In the flour and bran produced in the laboratory, the bran product was similar to that of the other samples and the endosperm was carefully selected to exclude bran and embryo (germ). About 40% of flour was recovered from each wheat. It was, of course, recognized that it was difficult to obtain a representative sample of the entire endosperm. The harder portions, richest in protein, are always nearer the bran. Possibly there is a difference in the relative proportions of the different proteins in the harder and the softer portions of the endosperm. For our purpose, at this time, it is not of great importance.

Results

In the results obtained on this series of brans, as shown in Tables I and II, it will be noted that there is a marked degree of similarity among all of them and that their general characteristics may be seen in the average results, especially when these results are applied to the

TABLE I

NITROGEN OF DIFFERENT COMPOUNDS AS FOUND IN BRANS FROM 12 MILLS USING DIFFERENT TYPES OF WHEAT. BRANS REGROUND TO FREE FROM FLOUR AND MADE TO PASS THROUGH A NO. 40 GRTIZ GAUZE. PER CENT NITROGEN IN BRAN CALCULATED TO DRY MATTER BASIS

Source of wheat	Form of nitrogen compound						Non-protein
	Total	Albumin	Globulin	Prolamine	Glutelin	Not dissolved by 0.2% caustic soda	
Minnesota and No. Dakota	2.67	0.61	0.39	0.27	0.64	0.50	0.26
Minnesota	2.77	.60	.40	.33	.69	.50	.25
Montana	2.61	.47	.36	.35	.67	.48	.28
Nebraska	2.64	.50	.38	.34	.58	.60	.24
Kansas	2.73	.58	.31	.30	.67	.62	.25
Oklahoma	2.61	.48	.41	.25	.65	.57	.25
Texas	2.57	.59	.36	.32	.57	.47	.26
Missouri	2.42	.47	.37	.22	.61	.52	.23
Illinois	2.39	.45	.33	.30	.55	.50	.26
Michigan	2.62	.48	.35	.47	.55	.53	.24
Washington	3.06	.59	.47	.47	.78	.48	.27
Durum	2.61	.58	.42	.32	.50	.54	.25
Average	2.64	.53	.38	.33	.62	.53	.25

TABLE II

NITROGEN OF DIFFERENT COMPOUNDS AS FOUND IN BRANS FROM 12 MILLS USING DIFFERENT TYPES OF WHEAT. BRANS REGROUND TO FREE FROM FLOUR AND MADE TO PASS THROUGH A NO. 40 GRITZ GAUZE. NITROGEN CALCULATED AS PER CENT OF TOTAL NITROGEN

Source of wheat	Form of nitrogen compound					Not dissolved by 0.2% caustic soda	Non-protein
	Total	Albumin	Globulin	Prolamine	Glutelin		
Minnesota and No. Dakota	2.67	22.9	14.6	10.1	24.0	18.7	9.7
Minnesota	2.77	21.7	14.4	11.9	24.9	18.1	9.0
Montana	2.61	18.0	13.8	13.4	25.7	18.4	10.7
Nebraska	2.64	18.9	14.4	12.9	22.0	22.7	9.1
Kansas	2.73	21.2	11.4	11.0	24.5	22.7	9.1
Oklahoma	2.61	18.4	15.7	9.6	24.9	21.8	9.6
Texas	2.57	22.9	14.0	12.5	22.2	18.3	10.1
Missouri	2.42	19.4	15.3	9.1	25.2	21.5	9.5
Illinois	2.39	18.8	13.8	12.6	23.0	20.9	10.9
Michigan	2.62	18.3	13.4	17.9	21.0	20.2	9.2
Washington	3.06	19.3	15.4	15.4	25.5	15.6	8.8
Durum	2.61	22.2	16.1	12.3	19.1	20.7	9.6
Average	2.64	20.1	14.3	12.4	23.5	20.0	9.7

per cent of the several forms of nitrogen expressed as per cent of the total nitrogen.

The proteins which are present in the largest amount are the glutelins. They amount on an average, to nearly one-fourth of the total protein. The albumin protein, and that protein not dissolved by dilute alkali, are equal and next highest in amount. The nitrogen of each of these fractions represents one-fifth of the total nitrogen. The globulins are somewhat higher in amount than the prolamines and their nitrogen is equal to one-seventh of the total nitrogen. The proteins present in the least quantity are the prolamines. Their nitrogen amounts to about one-eighth of the total. The amount of non-protein nitrogen is very uniform throughout and is nearly one-tenth of the total.

Comparing the results shown for flour, bran, and wheat, Tables III and IV, it is noted that the nitrogen in the flour is largely in the gliadin and there is very little albumin or globulin present. The soft wheat flour contains less than one-third as much glutenin as gliadin and the Marquis flour contains somewhat less than one-half as much glutenin as gliadin. There is about one-half as much globulin as albumin in the Marquis flour while in the soft wheat flour the amounts are equal and

TABLE III
NITROGEN IN PER CENT OF DRY MATTER AS FOUND IN DIFFERENT COMPOUNDS
IN A SPRING AND A SOFT WINTER WHEAT, AND IN PURE BRAN AND
PURE FLOUR SEPARATED FROM THEM

Material	Form of nitrogen compound						Non-protein
	Total	Albumin	Globulin	Prolamine	Glutelin	Not dissolved by 0.2% caustic soda	
Spring wheat	3.10	0.27	0.11	1.58	0.82	0.19	0.13
Flour	2.92	.17	.09	1.81	.78	.00	.07
Bran	3.12	.70	.50	.32	.73	.57	.30
Soft winter wheat	2.28	.20	.24	1.00	.61	.16	.07
Flour	1.91	.10	.10	1.29	.39	.00	.03
Bran	2.53	.48	.42	.41	.66	.52	.24

TABLE IV
NITROGEN CALCULATED AS PER CENT OF TOTAL NITROGEN AS FOUND IN DIFFERENT
COMPOUNDS IN A SPRING AND A SOFT WINTER WHEAT, AND IN PURE
BRAN AND PURE FLOUR SEPARATED FROM THEM

Material	Form of nitrogen compound						Non-protein
	Total	Albumin	Globulin	Prolamine	Glutelin	Not dissolved by 0.2% caustic soda	
Spring wheat	3.10	8.7	3.6	50.9	26.5	6.1	4.2
Flour	2.92	5.8	3.1	62.0	26.7	.0	2.4
Bran	3.12	22.4	16.0	10.3	23.4	18.3	9.6
Soft winter wheat	2.28	8.7	10.5	43.9	26.8	7.0	3.1
Flour	1.91	5.2	5.2	67.5	20.4	.0	1.7
Bran	2.53	19.0	16.6	16.2	26.2	20.5	9.5

about the same as the globulin in the Marquis flour. All nitrogen compounds in the flour are dissolved in dilute alkali and the amount of non-protein nitrogen is small. There is a consistent relation for the findings on the wheat between those for the flour and the bran.

In a recent circular of the United States Department of Agriculture, Jones (1931) has indicated a factor of 6.31 for converting the nitrogen of bran into protein, and based upon this has proposed the factor 5.83 for converting the nitrogen of wheat into protein. It will, therefore, be of interest to study the factor for bran in the light of the information shown in these results.

Osborne (1907) has given the nitrogen contents of gliadin, glutenin, leucosin, and globulin as the average results of a considerable number

of preparations which he has made and analyzed, apparently in a most thorough and painstaking manner. His findings are, apparently, a safe working basis for computing the proteins from these different forms of nitrogen as indicated. In Table V are shown the average

TABLE V
PROTEIN IN BRAN CALCULATED FROM THE NITROGEN OF DIFFERENT COMPOUNDS

	Nitrogen in proteins	Protein factor	Average nitrogen in bran	Calculated protein
	P.ct.		P.ct.	P.ct.
Albumin (Leucosin) ¹	16.80	5.95	0.53	3.15
Globulin (Edestin)	18.39	5.44	.38	2.07
Prolamine (Gliadin)	17.66	5.66	.33	1.87
Glutelin (Glutenin)	17.49	5.72	.62	3.68
Not dissolved by 0.2% alkali	²	5.7	.53	3.02
Sum			2.39	13.79
Non-protein nitrogen			.25	
Total nitrogen			2.64	
Total nitrogen \times 5.7				15.05
Difference due to non-proteins and possibly other causes				1.26

¹ The words in parenthesis are the names used by Osborne. The figures following are the average amounts of nitrogen which he found in those compounds. We have calculated the factor for protein from these findings. The undissolved protein is calculated from the undissolved nitrogen by 5.7.

² Unknown.

amount of these forms of protein nitrogen multiplied by the correct factor calculated from his nitrogen determinations. The albumin nitrogen multiplied by the factor 5.95 shows albumin 3.15%. The globulin nitrogen multiplied by the factor 5.44 shows globulin 2.07%. The gliadin nitrogen multiplied by the factor 5.66 shows gliadin 1.87%. The glutenin nitrogen multiplied by the factor 5.72 shows glutenin 3.53%.

It may be a question as to what factor we should use for the undissolved nitrogen. Since the protein nitrogen for all the other compounds are below 6, it would be somewhat surprising if we should find in the bran a protein which contains a much higher factor. However, if we use, for this form of nitrogen, the conventional factor 6.25, we shall have 0.53% multiplied by 6.25 or 3.31% protein. If we use the factor 5.7, we would have about 0.3% less. Taking the sum of the several forms of protein, including that of the undissolved nitrogen multiplied by 6.25, we have a total of 14.08% true protein. The average total nitrogen in these samples was 2.64%. This multiplied by 5.7, the commonly used factor for wheat, gives 15.05% crude protein. Subtracting from this crude protein the 14.08% shown in Table V as the sum of the true proteins, we have a difference of 0.97%. The sum of the true protein nitrogens is 2.39%, which is 0.25% less than the total nitrogen; and is from the non-protein nitrogen com-

pounds. It was pointed out by Teller (1932) that there is a wide difference in the nitrogen factor for the different forms of non-protein nitrogen compounds which have been found in wheat. We cannot readily take an average of these factors to show the amount of these non-proteins from the non-protein nitrogen present in bran, and we do not know their identity.

On the other hand, if we multiplied the undissolved nitrogen by the factor 5.7, we shall have 3.02% instead of 3.31%. This gives a total of 13.79% as the sum of the several protein compounds. It is interesting to note that the true protein nitrogen (2.39%) multiplied by the factor 5.7 gives 13.62%, which is but little below the 13.79% found as above. Based upon the above determinations, it is, to our minds, apparent that the factor 5.7 is entirely consistent as a protein factor for bran. Since it is also the recognized protein factor for flour and the factor is not materially different for the protein in the small amount of embryo, it naturally follows that 5.7 is the right protein factor for wheat.

Summary

Our study of the nitrogen compounds in a considerable number of brans, well-freed from flour, shows that the different forms of proteins in them are materially different in amounts and proportions from those in flour. There is relatively more albumin, globulin, and glutelins in the bran. While the gliadin nitrogen in the pure endosperm is often more than half the total nitrogen present, it does not constitute more than one-eighth the total nitrogen in the pure bran. The relative proportions of the several proteins found in the several brans are fairly constant, especially when considered as per cent of the total nitrogen.

There is in the bran an appreciable amount of non-protein nitrogen compounds. In the 14 samples examined, the proportion of non-protein nitrogen is very constant and amounts to nearly one-tenth of the total nitrogen present. While the nature of the non-protein nitrogen compounds has not been fully determined, the examinations made indicate that not more than one-fourth are in the form of amides of the asparagin type.

The results of this study appear to confirm the correctness of the factor 5.7 by which to multiply the nitrogen to determine the amounts of crude protein in bran and wheat.

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STATISTICS IN THE SERVICE OF CEREAL CHEMISTRY

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Introduction

It is gratifying indeed to those who labor for the increase of precision in scientific description and interpretation to receive from their fellows a request for the presentation of an address on the importance of the ideal being striven for. Such, at least, was my experience when I received the invitation of the Program Committee of this 18th annual convention of the Association to prepare a paper on the subject of the importance of statistical methods for cereal chemists.

The responsibility involved in speaking on the subject chosen for today, however, quite outweighs the gratification, and I confess to feeling somewhat of the burden that was Francis Galton's when he stated in his book "Natural Inheritance": "I have a great subject to write upon, but feel keenly my literary incapacity to make it easily intelligible without sacrificing accuracy and thoroughness." To be sure, the situation now is an entirely different one to that which faced that most inspiring master, for he had to present a concept that was slowly to revolutionize scientific thought, and his audience was sadly unfamiliar with even the most elementary principles of the mathematical analysis upon which his work rested. In part, at least, my audience is reasonably well acquainted with certain of those principles, and my present contribution is not to blaze a new trail, but to point out to you the wisdom of exploring for yourselves the security of paths which workers in other fields have followed with intensifying zeal.

My task weighs somewhat heavily, too, for the greatness of the subject which I must discuss has led many to attempt its application with a serious neglect of that *accuracy and thoroughness* which is vital to its existence. My message must be in part a warning against the abuse of statistical treatment. The power of statistical analysis is not infrequently being resorted to by individuals poorly equipped to employ it, and incorrect conclusions are drawn. Sometimes it appears to be employed as a means for the seeming glorification of data that are intrinsically deficient or without merit as representations of the phenomena they are purported to measure.

Historical Notes

While statistical analysis is a mathematical activity of comparatively recent creation, the compilations of data to which the term statistics was first applied extend back into antiquity almost as far as the path of recorded history goes. Impelled by motives of benevolence, fear or greed, monarchs of ancient time required numerical tallies of their wealth and strength. The usefulness of such official tabulations so commended itself with the march of civilization and particularly the growth of democracy that the practice has grown and vastly widened in scope. Careful studies by scholarly mankind of such official tabulations appear to have been made for the first time in Germany about the middle of the 16th century, and in 1660 such studies formed a part of the curriculum of the University of Helmstedt.

The term *statistik* appears to have been originated by the German scholar Achenwall, he deriving it no doubt from the mediaeval Latin, *status*, meaning the political state. The word was later adopted by the English workers, and was soon widely used to connote numerical tabulations of the natural resources, production, population, power and political circumstances of a country. However, the implications of the term have gradually changed in the last century from the particular materials to the general method of summarizing the relevant information provided in variable data. This change has been due, on the one hand, to the practical need of interpretation of variable data, irrespective of its origin, and on the other hand to the rapid development since 1800 of the mathematical theory of probability.

Serious studies in the theory of probability had their origins in a subject which gained for them much disapproval for a considerable period of time. The problem was that of the division of stakes in an incompleting game between two players of unequal ability. Pascal, discussing the problem in correspondence with Fermat in 1654, as the result of the enquiry of a gambler of eminence, generalized the theorem more and more until "it finally assumed the dignity of a mathematical concept of great importance" (Walker, 1929). He thus laid the foundations of the mathematical theory of modern statistics. Bernouilli was probably the first mathematician to conceive the importance of the application of the principles of probability to civil, economic and moral affairs. His eminence as a pure mathematician demanded considerable respect for the subject of his discussions, and he may be said to have weaned the theory of probability from its ill repute. The labors of La Place, the French mathematician, and Gauss, the German astronomer, added much to the development of the theory, the latter laying the foundations of its application in the precise sciences.

Much aid appears to have been gained from the mathematical knowledge of such men in the interpretation of official statistics, the logical blending of the mathematical theory and the analysis of practical problems thus having its inception. However, it was not till the enthusiasm and the life work of Quetelet and of Galton became focussed on that blending that the power of the *new mathematics* was felt in the applied sciences, and then not to any marked extent till death had taken them from their labors.

Just as lack among chemists of facility in mathematical reasoning caused the profound work of Gibbs to remain practically without recognition until after his death, so that deficiency in the training of scientists generally until recent years has hindered the appreciation of the power and practical utility of the statistical method of analysis. Biologists generally have shown an apathy and even resistance toward statistical thought that has been almost akin to a pathological phobia, while but few in the ranks of the biological chemists have taken steps to avail themselves of the fruits of the labors of the men comprising the biometric school. To these men, notably Quetelet, Galton, Karl Pearson and his associates at the Galton laboratory, and more recently Fisher, we owe, almost in its entirety, the theory and practical technique of the statistical method.

I venture the opinion that an appreciation of the value of this method of analysis in biochemical investigation will prove to be essential to the elevation of biochemistry to companionship with physical chemistry as a precise science. Indeed, with the increasing refinement and precision of measuring instruments, the problems of uncontrolled variation may be expected to become manifest in every field of science, and statistical analysis will necessarily be invoked to solve the problems incurred.

Problems of the Distribution of Variates

Unquestionably the function of applied statistics is the measurement of the characteristics of variable systems and the evaluation of the probabilities that in replicated experiments the characteristics measured will not deviate from an established value by more than a certain amount. The governing conditions in practical cases are those wherein the particular properties of a sample drawn from a variable system are known and it is desired to determine the characters of the parent system, or population. Analysts A and B repeatedly and independently determine the amount of ash in a given sample of flour. Variability—temporarily beyond control—characterizes both series of results. The questions arise "What is the true amount of ash in the flour?"—"Are the two series of results in essential agreement with one

another?" Such a case is representative of the type of problems for which that branch of mathematical reasoning known as statistics has been developed. It is impossible to answer either question with certainty. It is possible by applying the techniques of statistics to determine the probability attaching to any given answer to each question. The event of greatest likelihood might then be selected as the best estimate of the true measure of the character. The process of reasoning is one of induction, and precisely determinable probability must form the basis of judgment.

In an earlier paper (Treloar, 1931), I have defined a few of the simpler statistics that have proven to be of wide service in the description of variable quantities, and have discussed in a preliminary way some of the characters of commonly occurring distributions of frequency. It would be advantageous at this time to amplify certain of those concepts, since they form the major section of the foundation of the entire statistical structure.

Just as the ideas of early mankind concerning the universe were revolutionized when the Greeks discovered that the stars moved in an orderly manner, so scientists of later days found fertile fields of investigation expanding in unlimited view before them when Quetelet, the great Belgian mathematician, astronomer and scientist of the last century, blazed the trail through the previously unrelenting mountains and showed that variation in the physical and biological universe was not chaotic but had beautifully orderly characters—an orderliness of distribution that had been known for some time to be typical of measurements of stellar phenomena. It was a discovery of profound possibilities indeed that biological variation could thus be expressed with the precision and clarity provided by simple mathematical formulae. Writing of the phenomenon of variation as depicted particularly by the "normal" curve, Francis Galton expressed himself in the words:

"I know of scarcely anything so apt to impress the imagination as the wonderful form of cosmic order expressed by the 'law of error.' [It] would have been personified by the Greeks and deified, if they had known of it. It reigns with severity in complete self-effacement amidst the wildest confusion. The huger the mob and the greater the anarchy the more perfect is its sway. Let a large sample of chaotic elements be taken and marshalled in order of their magnitudes, and then, however widely irregular they appeared, an unexpected and most beautiful form of regularity proves to have been present all along."

Although orderly variation may be expressed in a truly endless variety of forms, those forms lend themselves to fairly simple classification. While generalized description of them may readily be made in terms of symmetry, modality, range and relative concentration about central values, it is highly advisable to have an objective classification of universal applicability, such has been provided by Karl Pearson in

terms of the moments of the systems. This classification has proven of such wide service in statistical definition that the seven Pearsonian types together with the normal curve form a widely accepted basis for the description of frequency distribution. Cereal chemists have not been made familiar with this classification to any important degree, largely because the statistical problems demanding immediate consideration have been chiefly those of association and differentiation. However, all statistical analysis revolves about the manifold problems of distribution, and discussions concerning differences between statistics only becomes possible through a knowledge of, or acceptance of hypothesis concerning, the distribution of variates.

One phase of the enquiry concerning the distribution law of variates in which cereal chemists have taken an active interest is that of the distribution of error in making certain purely chemical analyses or in making more artful determinations such as that of loaf volume. In the former work, the American Association of Cereal Chemists, through its Committee on Methods of Analysis, has undertaken a pioneer work of very great importance to the development of its own special interests and to the advancement of precise sciences generally. In seeking to define a *law of error* through extensive collaborative check testing, cereal chemists are giving a much needed emphasis to a fundamental principle, namely, that the precision of any complex evaluating mechanism can only be accurately and completely defined by extensive *practical tests*.

Illustration of this point may be given by reference to data relating to the evaluation of loaf volume. The three histograms in Figure 1 have been presented in an earlier paper (Treloar and Larmour, 1931) as illustrations of the variation in the volume of loaves baked by three operators in series of 50 replicates from the same sample of flour. Limits of accuracy in replicate baking tests were then suggested on the hypothesis that the "normal" law of error was the appropriate law in each case. The adequacy of that hypothesis may be investigated statistically by means of Pearson's (1900) powerful objective criterion, the χ^2 , P test. The probabilities that the given distributions in each case satisfy the normal law are given in the left section of Table I.

The "fit" of the normal curve to the data of bakers B and C is apparently very satisfactory, greater discrepancy between the curve and the histogram in each case being expectable 6 times in 10 through chance factors of sampling only. For the data of baker A, the fit is not so good, but the disagreement is not large enough to consider the curve to be demonstrably unsatisfactory as a plausible graduation of the data.

The feasible suggestion that some form of distribution other than the "normal" law might provide a better hypothesis has led to the fit-

ting of the curves of the Pearsonian system suggested by the moments of the distribution. While the χ^2 , P criterion applied to the new hypotheses shows distinct improvement in the case of baker A, no greater precision or advantage seems to have been gained on the whole over

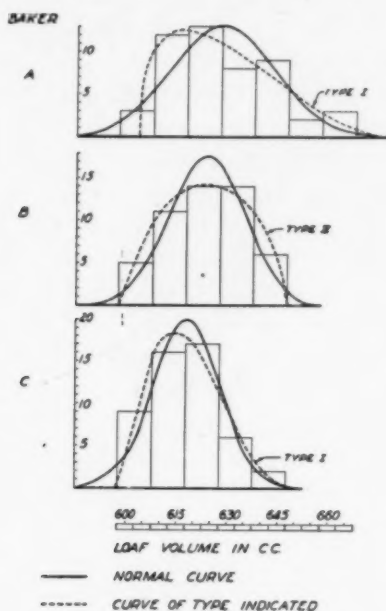


Fig. 1. Histograms and fitted curves for the distribution of error in the determination of loaf volume (Data from Treloar and Larmour, 1931).

TABLE I
CRITERIA OF THE GOODNESS OF FIT OF THE CURVES PRESENTED IN FIGURE 1

Baker	"Normal" curve			Personian type	Other curves		
	d/f^1	χ^2	P^2		d/f^1	χ^2	P^2
A	4	7.30	.12	I	3	2.11	.55
B	2	0.99	.60	II	2	0.29	.87
C	2	0.92	.64	I	1	0.77	.40

¹ Degrees of freedom.

² From Elderton's table (Pearson, 1925).

the normal curves when the two other cases are considered. For baker B the Pearson type II curve is more closely in agreement with the empirical data to but a slight degree, while for baker C the type I curve as suggested by the moments shows a poorer fit. In all three cases the "a-normal" curves show an abrupt limit to negative errors in every case and different degrees of abruptness for the limits of high

positive errors. These features seem hardly satisfactory and are difficult to justify from practical considerations.

Following the scientific principle of generalization that, of two alternative hypotheses of reasonably equal merit the simpler should be chosen, it seems clear that the "normal" curve should be accepted, *pro tempore*, as providing the most suitable graduation of error in these cases.

This, then, might serve to illustrate something of the nature of the problems of distribution of individual measurements which cereal chemists are attempting to solve and which they must face, for the philosophy of the fugitive ostrich is not a comfortable one for more than a short period of time. The hypothesis of normality of distribution of variates that can with confidence be accepted as being approximately correct until more extensive study will enable its replacement by factual description of greater refinement, will save many a blunder that might otherwise be made without it.

It has been a direct result of the serviceability of the normal curve that the standard deviation has been so generally accepted as the measure of dispersal, for it is a much more efficient statistic in defining the curve than is the average deviation, which is somewhat easier of computation. A measure of dispersal of perhaps even more universal service in *numerical work* is the square of the standard deviation, known as the *variance*. The latter, however, suffers the limitation of summarizing as a second order magnitude, a character which is in itself linear, and thus for many purposes has not the appeal which its square root possesses.

Those who do not differentiate between the problem of the statistical description of the sample and that of estimating the population character from the sample, notably Fisher and his associates, have insisted on the introduction of a formula for the standard deviation which is not in accord with the definition given originally by Pearson. I refer to the formula for "small samples,"

$$s_z = \sqrt{\frac{\Sigma(x - \bar{x})^2}{(N - 1)}} \quad (i)$$

$$= \sqrt{\frac{N}{(N - 1)}} \sqrt{\frac{\Sigma(x - \bar{x})^2}{N}}. \quad (ii)$$

The use of $(N - 1)$ in expression (i) instead of N (as given by Pearson) aims to provide a correction for the average error in \bar{x} (the mean of the sample). Since the moments are necessarily taken about the sample mean, \bar{x} , and not about the population mean (which is unknown), the average standard deviation of samples will necessarily be somewhat

less than the standard deviation of the population, the error decreasing as the size of the sample increases. Although this has been a familiar fact to mathematicians and astronomers since the time of Gauss at least, it was not recognized as it might have been by biological workers; however, the difference was quite unimportant except for the case of "small samples."

The use of $(N-1)$ as above provides a valid correction *on the average* in the estimation of the *population variance* from the second moment of the sample. Thus if s^2 be the variance of samples of size N , and σ^2 the variance of the population from which the samples are drawn, then

$$\sigma^2 = \bar{s}^2,$$

where

$$s^2 = \frac{\Sigma(x - \bar{x})^2}{(N - 1)}.$$

Apparently it is not clearly understood by many users of equation (i) that it does not provide necessarily the *best* estimate of the population standard deviation. Indeed the formula is hard to justify, philosophically, when applied to a unique sample, which is commonly the case. Its use may only be tolerated on the basis that it provides a better *estimate* than when N is employed. However, the *exact* corrections applicable to the two common hypotheses concerning the nature of a unique sample have long since been provided by Pearson (1915).

After considerable contemplation of the problem from the point of view of both teaching and research, I have concluded that it is most desirable to clearly segregate the description of the sample from the problem of estimating the population parameters from the sample statistics. Such an attitude not only paves the way to an intelligent understanding of statistical procedures by those not fully conversant with the mathematical details of the subject, but it also provides the same equational definition of both the parameter and the corresponding statistic. To return to the case in point, it seems to me to be highly desirable that the term *standard deviation* should be applied solely to the square root of the mean second moment of a series of values about their own mean. Thus

$$\sigma_x = \sqrt{\frac{\Sigma(x - \bar{x})^2}{N}} \quad (\text{iii}),$$

as originally defined by Pearson. The corrective factor appropriate to the particular problem of estimation may always be applied more clearly and logically as the second step when such commends itself in the analysis.

There is not any statistic which commands a more prominent place in analyzing the problems of distribution or differentiation than the standard deviation, or its equivalent measure, the standard error. I feel that no remark of apology is needed for the time given to it in the present discussion of distribution.

The Measurement of Association

Studies of the variation in characters considered singly leads naturally to enquiries concerning the tendency of related characters to show associated variation. It is desirable to touch briefly on this subject, which has taken a prominent place in statistical discussions of cereal-chemistry data.

Francis Galton's conception of partial causality and the measurement of the degree of interdependence between things on a universally comparable scale was one of momentous importance to science. Without question the significance of the concept far eclipses the immediate importance of the coefficient of correlation which he suggested as a satisfactory technique for fulfilling the requirement. It was responsible probably more than anything else for attracting to the field of applied statistics the genius of Karl Pearson whose summary (Pearson, 1930) of the principle visualized by Galton is worthy of full quotation:

"Galton, turning over two different problems in his mind, reached the conception of correlation: *A* is not the sole cause of *B*, but it contributes to the production of *B*; there may be other, many or few, causes at work some of which we do not know and may never know. Are we then to exclude from mathematical analysis all such cases of incomplete causation? Galton's answer was: 'No, we must endeavor to find a quantitative measure of this degree of partial causation.' This measure of partial causation was the germ of the broad category—that of correlation, which was to replace not only in the minds of many of us the old category of causation, but deeply to influence our outlook on the universe. The conception of causation—unlimitedly profitable to the physicist—began to crumble to pieces. In no case was *B* simply and wholly caused by *A* nor indeed by *C*, *D*, *E*, and *F* as well! It was really possible to go on increasing the number of contributory causes, until they might involve all the factors of the universe. The physicist was clearly picking out a few of the more important causes of *A*, and wisely concentrating on those. But no two physical experiments would—even if our instruments of measurement, men and machines, were perfect—ever lead to absolutely the same numerical results, because we could not include all the vast range of minor contributory causes. The physicist's method of describing phenomena was seen to be only fitting when a high degree of correlation existed. In other words he was assuming for his physical needs a purely theoretical limit—that of perfect correlation. Henceforward the philosophical view of the universe was to be that of a correlated system of variates, approaching but by no means reaching perfect correlation, i.e. absolute causality, even in the group of phenomena termed physical. Biological phenomena in their numerous phases, economic and social, were seen to be only differentiated from the physical by the intensity of their correlations. The idea Galton placed before himself was to represent by a single numerical quantity the degree of relationship, or of partial causality, between the different variables of our ever-changing universe."

The chemist, possibly just as much as the physicist, has profited in the building of his knowledge in the past by the use of the concept of complete causation. Results have been observed to flow directly

from, and in simple relationship to, the operation of forces or interaction of materials. Boyle was able to announce his most important gas law through careful observation of only three major variables which lent themselves to experimental control. However, imperfections of his prediction equations remained unexplained until the mathematical development of the kinetic theory of gases showed that three variables were not sufficient to completely define causation in the volume changes of a gas.

A vastly wider field is thrown open to the view when it is realized that the ideal of complete causation between *A* and *B* presents but the limiting case: that other forces at play act as modifiers to varying degrees, causing the results to approximate only to complete interdependence. The situations in which these "modifiers" exercise an influence so gross in magnitude as to noticeably shroud the true relation between *A* and *B*—situations which cereal chemists are constantly confronted with—are those to which the methods of correlation analysis have customarily been applied, with profit proportional to the investigators understanding of the principles involved.

That a relationship exists between the protein content of flour and the volume of the loaf of bread it will produce under standard conditions has been known in a general way for a long time. In statistical terminology the former was known to partially define the causative system of the latter. However, a quantitative measure of the intensity of the association was not made available until Zinn's memorable work was published in 1923. His coefficients of correlation (omitting the faulty coefficient for North Dakota spring wheats) varied over a range from $+0.26$ to $+0.80$. He did not concern himself with tests for the significance of the differences between the coefficients; he was essentially concerned with "the positive and generally high correlation between loaf volume and protein in flour." Many more such coefficients have been calculated since then, so that today, in my own records alone, I can find any size coefficient I want for this relationship, from essentially zero to well nigh unity.

The question "What intrinsic relationship is there between protein content and loaf volume?" might thus appear to have received an answer in the form of correlation coefficients, little if at all superior to that available over 20 years ago. However, careful statistical examination of the coefficients will give convincing proof of the existence of a condition of the greatest importance, that of heterogeneity characterizing the various series of data. The relationship between protein content and loaf volume can be so modified by varying sources of the grain, fluctuating the milling procedure, or modifying the baking technique, that an estimate of the correlation under one set of conditions may be quite

different from that characterizing other conditions. This is but practical evidence concerning a familiar principle to the statistician, namely, that a definition of the conditions of any experiment producing data embraces a definition of certain potential modifiers to the intrinsic relationship being studied. This is so generally true that in all statistical investigations a definition of the conditions which determine in any sense the magnitudes of the data being studied is essential to the interpretation of the final statistics reached.

The effect of all measurable influences determining or associated with an end result may in turn be subjected to evaluation by the correlational method. By means of such techniques as that of partial correlation, equations for prediction can finally be established giving due weight to each measured factor. When all factors of influence on the final result are known, measured and accounted for in the equation, then perfect prediction becomes possible.

When perfect prediction capacity is attained in any formula, then surely it must be accepted that the causative system is fully defined, no matter how indirectly that be. It is in this sense that the statistician can speak of complete and partial causation, meaning complete or partial predicting power. This must not in any sense be interpreted as meaning that correlation between two variables implies causation in the more colloquial sense of that term. Although protein content of flour may undoubtedly be used to define in part the causative system determining loaf volume, it cannot be considered as a corollary that an increment of protein will, of itself, cause the corresponding increment of change in loaf volume. That is a problem of a different character.

Having defined, then, the concept of correlation, a few remarks concerning the serviceability to the cereal chemist of the various techniques available for measuring it may be in order.

Akin to chemists generally, the cereal investigator has at his disposal many methods of a highly sensitive character which measure *quantitatively* and with considerable precision the magnitude of constituents or masses. On the other hand, many characters such as crumb texture or crust color cannot be measured satisfactorily and must be described by broad classification which, in turn, may or may not form a well defined continuum. The measurements of other characters would logically fall in intermediate groups between these extremes. It will be desired to include consideration of all such variables in the study of interrelationships. The measurement of association between them will present situations of distinctly different types and necessarily demand the employment of different statistical techniques. Almost entirely through the genius of Karl Pearson, several such techniques for measuring correlation are available. While discussion of the use-

fulness and limitations of these methods of measuring association, together with illustration of their application, must be reserved for another occasion, it is worthy of note here that, as yet, correlation analysis in cereal chemical studies has practically been confined to situations to which the Galtonian coefficient r , or its partial and multiple analogues, is applicable. The correlation ratio, contingency, bi-serial and tetrachoric coefficients, etc., of Pearson, should vastly extend the power of the cereal chemist in measuring influences in the causative systems with which he is concerned.

The coefficient of correlation, which is valid solely for those cases where the line of average relationship is truly rectilinear, is by all odds the best known measure of association to practical scientists, and has been widely used in cereal chemical studies, chiefly for the purpose of comparing intensities of association. Its use has served to remove from the realm of controversy the relative merits of various bases of prognostication, and has clearly shown that many previously accepted foundations of specific prediction have been over-rated in importance.

A failure to grasp the essentials of interpretation has led some into erroneous deduction, while the significance of dangers which intensify rapidly as the number of cases investigated is reduced to small numbers are realized by too few. The statistical significance of a correlation coefficient, i.e., its deviation from zero as possibly reflecting solely an error of random sampling, has in several instances been all too cursorily examined; formulae valid only for large samples have been applied to very small numbers with consequent false conclusions. While such errors become exposed in time, they are apt to lead temporarily to false enthusiasms and large expenditures or sacrifices that later may be bitterly regretted when the inexcusable error is revealed.

That which is obvious to the careful thinker, but which is sometimes overlooked in the rush of enthusiasm of those who find themselves on the verge of discovery, is the principle that generalization from a sample can only be valid for that population of measurements of which the sample is a random selection. The factors which might cause heterogeneity among samples must always be given careful consideration when the scope of generalization is being established. A case in point may be cited. Canadian workers appear to have demonstrated beyond any doubt that the use of the bromate differential test intensifies the relation between loaf volume and protein content, whereas cerealists in the United States, using even the same wheat varieties grown under different conditions, not infrequently find the opposite results. It would appear, then, that *for wheats grown under certain northern conditions on this continent*, the introduction of bromate into the baking formula reduces the intensity of the modifiers to the relationship be-

tween protein content and loaf volume. The problem to establish the relation of this effect of bromate addition to geographic origin of the wheats remains unsolved except in a very general way. It is clear, however, that discussions of the value of the bromate differential test must take into account the factor of heterogeneity of the edaphic and climatic environment governing development of the grain. In the early stages of the discovery of the importance of the bromate differential test, this point seemed to be overlooked by some workers entering the discussion.

In the logical advance from measuring degree of association to prediction of variable characters, little more than preliminary scouting has been done in the field of cereal chemistry so far. Such work has practically been confined to the prediction of loaf volume, with results that give encouragement and yet raise a notice of warning to the enthusiasts who would speculate on slight margins of protein content.

The final objective for which the scientist labors is that of permitting the formulation of valid generalizations concerning the forces which, in varied form, constitute the universe. The generalizations are expressed in scientific laws which find their ultimate precision in mathematical formulation. Such laws are simply prediction equations of great accuracy. They are possible of attainment in cereal chemistry through the patient labors of those who will give exhaustive study to the problems of prediction—the logical outgrowth of correlation analysis.

On the Establishment of Significant Differentiation Between Statistics

It is an inevitable consequence of variation among the individual measurements (or *variates*) comprising samples that summarizing functions of the variates will themselves vary from sample to sample despite a common origin of those samples. The simplest of such summarizing functions (which are commonly known now as *statistics*) is the mean, or measure of type, and there is no more important section of statistical analysis than the evaluation of the significance of the differences between means calculated from samples of different origin. The identical problem exists with regard to all other statistics, such as the variance or correlation coefficient, and the procedure is essentially similar in all cases.

Every statistic calculated from a sample is essentially the basis of an estimate of the value of the corresponding function (known as a *parameter*) of the population from which the sample can be regarded as being randomly drawn. Thus, the unknown parameter is invariable while the statistics of samples vary about it as a more or less central value. This variation of the statistics is related in a simple

manner to the variation of the individuals comprising the population. Knowledge of the latter will define the former. Since the standard deviation of the sample usually provides the only measure available of the variation in the population, the desirability of securing high dependability in this measure is surely manifest, and the danger attendant upon the use of small samples is clear.

From the variability of the individuals (measured by the standard deviation) an estimate may be made of the variability to be expected in the statistics if they could be replicated. This latter variation is commonly computed as a *standard error* or *probable error* (P.E. = .6745 S.E.). It is upon a knowledge of these "errors" that the interpretation of the differences between statistics depends.

The problem of establishing real differentiation between like statistics from different samples would be, for the most part, one of insoluble complexity if complete certainty were to be demanded in the verdict. However, answers of great practical utility may be provided in terms of probability, which after all, forms the basis of practically all human decisions. There is much truth to the maxim: "One is certain of nothing in this universe."

The principle of the mathematical method of determining the significance of the difference between two statistics is capable of simple and succinct statement. The question which the statistician attempts to answer in each case may be stated as follows: "If the two statistics have arisen by random selection from *undifferentiated* populations with regard to the character in question, in what proportion of cases might a difference as great as that observed have so arisen?" If the probability evaluated accordingly should prove to be sufficiently small, i.e., below an accepted *level of significance*, the conclusion would be drawn that the hypothesis of no differentiation was unlikely enough to warrant its rejection.

Probability is customarily expressed by the mathematician on the scale of zero to unity and is usually symbolized by the letter P (or p). This scale is of such great utility, particularly for computational purposes, that any hurdle provided by informal convention would profitably be crossed to reach it. The most generally accepted level of significance is $P = .05$. A value of P below .05 would mean that there was less than 1 chance in 20 of the observed difference being due to the errors of random sampling only, and hence the odds become 19 or more to 1 that some factor other than sampling errors has contributed to the difference between the two statistics being compared.

All tests for the significance of the difference between statistics or variates call for the calculation of quantities such as the ratio of the difference to its standard or probable error, or "Student's" t function

for means of small samples, or Fisher's z function for correlation coefficients, etc., which values may be used as the argument in entering tables of the appropriate probability integral. The provision of such tables, notably by the Galton Laboratory (Pearson, 1925, 1931), and in the various writings of Fisher, represent much labor for the saving of onerous mathematical computation by others. With their aid and the use of the correct formulas for ascertaining the argument, it is a comparatively simple matter today to determine with adequate precision the probability of significant differentiation between statistics. Thus these two volumes of tables by Pearson (*loc. cit.*) and those published collectively by Fisher (1930) form almost indispensable reference sources on the computing desk.

I shall have to reserve for another occasion the elaboration of criteria of differentiation. Suffice it to state here that some of the tests appropriate to large samples have been discussed in these pages (Treloar, 1928). One is impelled to draw attention also to the lucid explanation by Goulden (1932) of the analysis of variance suggested by Fisher and now widely used in biological research, also to the many examples of statistical tests of differentiation in the writings in this journal, particularly of Geddes, Larmour, Bailey, Harris, and also the author.

A Note on Small Samples

With the provision in recent years of methods of statistical induction appropriate to the analysis of samples of small size, a tendency favoring small scale investigation has clearly grown in the ranks of many workers. Economic factors must be expected to limit the extent to which the replication of measurement or experimentation can be conducted, and it is entirely logical to restrict that replication to such a minimum as will still permit of the establishment of the facts for which the enquiry has been conducted. However, it is well to bear in mind that with small samples it is all but impossible to determine with accuracy the form of the frequency distribution of the variates. Hence it is necessary to adopt an hypothesis to meet the deficiency. Small sample theory is based accordingly upon the assumption of normality of distribution of the measurements comprising the sample, and is completely accurate only when that condition is fulfilled.

The emphasis given since "Student's" work (1908a, b) to the development of correct techniques for analyzing small samples has been the outcome of recognition of the error which is apt to result when methods devised for and correctly applicable to large samples are carelessly employed for the statistical analysis of small numbers of individuals. The mathematical problem was one of avoiding approxi-

mations in the theory such as had been made with complete validity under the condition that the sample was not below a certain ill-defined limit of smallness.

The question is not infrequently asked in connection with statistical interpretation, "In descending the scale of whole numbers, where does the large sample end and the small sample begin?" The necessary answer that there is no clear line of demarkation is not a very helpful one. It is worth while, then, to explain that the techniques which are valid for small samples are valid for *all* samples. However, they are somewhat less direct of application than those devised for large samples, and do not show any appreciable refinement over the latter when the number in the sample is, say 30 or more (for means). Serious differences will frequently appear, however, when N is less than 20, and for these small numbers it is not valid to use large sample theory. It is to the subject of "small samples" that Fisher has largely devoted his efforts in statistical theory. His text-book (1930) provides a condensed statement of that most valuable work.

Readings in Statistical Analysis

While in large part the mathematical theory of statistics is readily available in text-books of varying degrees of merit concerning their completeness, a notable deficiency exists in a clear exposition of the principles of statistical analysis for the research worker of meagre mathematical training. Two volumes aiming to meet this need have appeared in recent years, the first being by Fisher (1925, 3d ed. 1930). This has been followed by a text of considerable promise by Tippett (1931). Of these, the latter is of simpler statement and more comprehensive in scope, giving exposition of and full reference to the classical contributions of Karl Pearson and others to the general body of statistical knowledge.

As is the case in almost every branch of knowledge, students following the text book will lose a great deal by not consulting the original literature wherever possible. Unfortunately that literature is relatively inaccessible to most cereal chemists and they must therefore depend upon such secondary sources as happen to come within their reach. The careful reader of such pedagogical sources will endeavor to sense discussions of controversial topics and to defend himself as far as possible against excessive imbibition of the biases of the writer.

Conclusion

At the close of so generalized a discussion, one may perhaps be permitted to comment that the statistical leaven first introduced into the cereal-chemical lump by Roberts (1910, 1921) and by Zinn (1923) is

now working actively. Although the "expansion of the dough" has for many years been slow, localized and discouraging to those who labored "at the kneading," there is apparent today a wide recognition that statistical analysis is a most powerful accessory to scientific enquiry. The need now is rather for appreciation of the fact that while statistical computation may be accomplished in a routine manner by following given formulas, the interpretation of the end results calls for the use of logic that springs only from clear understanding.

In the development of any science some blunders will be made, and crude and irregular trails blazed into the unknown may be expected to precede the level highways upon which those of later generations may travel. So in the development of statistical thought we may expect increasing refinement as the more privileged surveyors follow the frontier pioneers. All modern statistical thought is not infallible. The processes of testing will continually proceed, with the removal of imperfections and the substitution of more perfectly consistent systems. These evidences of past imperfection, being rectified from within, must not be confused with that entirely different category of imperfect structures synthesized by abuse of the statistical method in the hands of those who fail to grasp its true function and interpretation. May cereal chemistry be spared the embarrassment of statistical blunderings among her workers.

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AN AUTOMATIC METHOD FOR MEASURING GAS PRODUCTION AND EXPANSION IN DOUGHS¹

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(Read at the Convention, May, 1932)

The attention of cereal chemists was drawn to the dual nature of flour strength by Wood (1907). He indicated that gas production shared importance with gas retention. The former was seemingly associated with enzyme phenomena, the latter with colloidal properties of the dough. Moreover, the production of gases of fermentation can be manipulated somewhat at the hands of the biochemist and there brought under control, particularly when it is initially at a low level.

Numerous methods have been suggested for the observation of the rate of gas production in a fermenting dough. The laboratory to which we are attached has been interested in these developments for nearly two decades. Bailey (1916) described an *expansimeter* which facilitated observations of gas production in an expanding dough. Bailey and Weigley (1922) determined the relation between the production of gas in and loss of gas from the fermenting dough. Their somewhat cumbersome method was simplified by Bailey and Johnson (1924). Even in this improved form the method is laborious, requiring practically constant attention from the technician to insure that no appreciable changes occur in gas pressure.

Using the rate of expansion of dough plus the gas leaking from the dough as a convenient expression of gas production, it is necessary that both temperature and pressure be maintained at a constant level. Jago (1911) and certain others who used a simple water displacement gasometer, attended to the control of temperature but apparently did not maintain a constant gas pressure. In the Bailey and Johnson device, the pressure was controlled by continually changing the position of the gas burette in such a manner as to thus compensate for any pressure changes which would otherwise result. This is a laborious business and one which every technician using this procedure would doubtless be glad to avoid. The method is coming to be of such service in various phases of flour strength studies as to justify the effort to render it essentially automatic. Moreover, the saving in

¹ Paper No. 1105, Journal Series, Minnesota Agricultural Experiment Station.

time of the operator will justify the added cost of the entire device. An hydraulic system adapted to this purpose was accordingly designed and placed in service by us, and this is diagrammed in Figure 1.

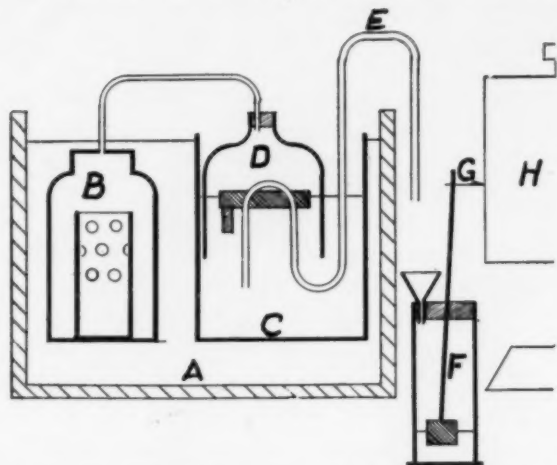


Fig. 1. Section of the automatic gas burette. (A) Water thermostat; (B) Sealed mason jar containing the dough; (C) Battery jar containing water overlaid with petroleum oil; (D) Bell jar supported by a clamp (not shown); (E) Siphon attached to the float in the bell jar; (F) Cylinder to receive water from the siphon; (G) Stylus attached to the float in the cylinder; (H) Kymograph drum.

In this constant pressure gas burette, *A* is a water thermostat; *B*, the fermentation jar as described by Bailey and Johnson (1924); *C*, a battery jar containing water overlaid with a film of oil to prevent absorption of carbon dioxide; *D*, a bell jar extending down into the water in the battery jar. The bell jar is connected to the fermentation jar by pressure tubing. A water manometer can also be connected to the bell jar if desired. Fastened to a balanced float inside the bell jar is a siphon *E* which extends from the lower portion of the battery jar and has its discharge end outside of the water bath and about 5 mm. below the water level which is the same in the bell jar and the battery jar. This distance is just sufficient to overcome the surface tension of the water. When the dough in the fermentation jar *B* expands or discharges gases of fermentation, the air displaced creates pressure in the bell jar *D* which lowers the water level in it and raises the water level in the battery jar *C*. As the siphon floats on the water in the bell jar, this lowering of the level in the latter causes the siphon to flow. It will continue to flow until the gas pressure in the bell jar is reduced to atmospheric pressure. In the present set-up, with a bell jar 10 cm. in diameter, a battery jar 15 cm. in diameter, and a siphon tube 2 mm. in diameter, a pressure equivalent to 2 mm. of water head is required to start the siphon flowing, and it will continue to flow until the gas pressure in the system approaches that of the atmosphere within the

equivalent of 0.5 mm. water head. It is thus evident that the changes in gas pressure in the system are insignificant for all practical purposes.

The water delivered from the siphon is proportional but not equal to the air displaced within the system by the fermenting dough. This water is collected in a cylinder shown as *F* in Figure 1. A float on the surface of the water in the cylinder supports a stylus which registers its level on the chart attached to the rotating kymograph drum. The apparatus is calibrated by passing known quantities of air or water into the fermentation jar *B*. A ruled chart showing the relation between the level of water in the cylinder *F* and the volume of displaced air can easily be prepared, and the curves drawn on the kymograph drum can then be read by superimposing the latter upon the standard chart and illuminating from behind.

With a little ingenuity, it is possible to arrange several such cylinders *F* about a single kymograph, thus taking off several records with one such device. Different colors of ink in the several stylus pens will facilitate distinguishing between the several curves. A proper selection of cylinders *F* of appropriate diameter will insure that the curves follow within the vertical limits of space provided on the kymograph drum that is used.

In Figure 2 several typical curves traced by this instrument are shown. Curves *A*, *B*, and *C* are from doughs representing 20, 15, and

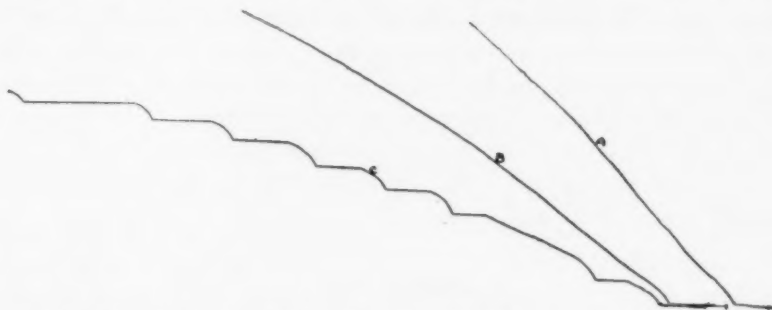


Fig. 2. Results of tests made with doughs representing 20 gms. (*A*); 15 gms. (*B*); and 10 gms. (*C*) of flour, respectively.

10 grams of the same flour, respectively. These curves show that the instrument records values proportional to the quantity of gas produced. The stairstep appearance of curve *C* is due to the rate of gas production being insufficient to keep the siphon in constant operation. However, if the points at which the curve becomes horizontal are connected, an approximately true curve results. The steps only represent 10 to 15 cc. of gas. Curves *D* and *E* in Figure 3 are replicates of *B* and show the close replication of work possible with this apparatus. The maximum difference recorded at any point on these two curves is less

than 10 cc., and the average difference is still smaller. These differences are of the same order of magnitude as have been observed with duplicate determinations made through the use of the old style gas burette.

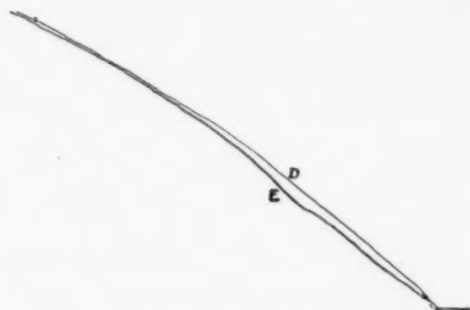


Fig. 3. Results of two replicated determinations.

Summary

The device previously described by Bailey and Johnson for measuring the expansion of doughs has been converted into an automatically registering mechanism. Air displaced by the expanding dough is conducted into a bell jar partly filled with water on the surface of which a buoy is floated. To the buoy a siphon tube is attached, the outlet of which is exterior to the bell jar. The water in the bell jar is in hydraulic equilibrium with water in an outer jar. As air enters the bell jar, the water in it, and hence the buoy, is lowered, and the siphon discharges water until the hydraulic balance is restored. Thus, there is no substantial change in gas pressure in the system. The discharged water is proportional to the volume of displaced air and can be readily recorded on a kymograph chart by collecting the water in a cylinder equipped with a float and stylus.

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THE ACID AND IODINE NUMBERS OF THE OIL FROM SOFT WINTER WHEAT ¹

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(Received for publication June 27, 1932)

Introduction

The iodine number of an oil or fat indicates the degree of unsaturation of the glycerides present. These unsaturated glycerides may combine with oxygen to form peroxides which by complex reactions decompose into compounds possessing a rancid odor and taste.

If there is considerable variation in the iodine number of different wheat oils, it would appear as though a determination of the iodine number might furnish a means of predicting the relative susceptibility of different wheats to the development of rancidity.

Since there is very little data available on the iodine number of wheat oils, it seemed worth while to determine the iodine number of a large number of wheat oils.

Coleman (1929) recently reports that the free acid content of wheat oil may also be an indication of the soundness of the wheat.

Historical

A survey of the literature reveals very little information in regard to the iodine number of wheat oils. Bailey (1925) quotes Spalth, and de Negri, Fabris, and Plucker on this subject. Ball (1926) also reports determinations of the iodine number of wheat flour oil. Bailey (1925) also quotes de Negri, Frankforter and Harding, and Alpers and Ball with respect to similar determinations on wheat embryo oil. The results of these investigators appear in Table I.

TABLE I
THE IODINE NUMBER OF FLOUR AND EMBRYO OIL FROM WHEAT

Investigator	Spaeth ¹	deNegri, Fabris and Plucker ¹	Ball	deNegri ¹	Frank- forter and Harding ¹	Alpers ¹	Ball ¹
Iodine number of flour oil	101.5	96.1-112.5	105.43	—	—	—	—
Iodine number of wheat em- bryo oil	—	—	—	115.17	115.64	122.6	123.64

¹ Bailey, 1925.

¹ This work was supported by a Fellowship from The National Soft Wheat Millers' Association.

Experimental

For the study of the iodine and acid numbers of wheat oils, samples of wheat from the 1929, 1930, and 1931 crops were obtained from representative mills located in the soft winter wheat producing areas of the eastern and central states. The majority of the mills sent in samples of locally grown wheat but a few sent in their regular mill mix which was a blend of soft wheat from several states. In the years 1930 and 1931, samples of pure varieties of soft winter wheats were obtained from several State Agricultural Experiment Stations in addition to those obtained from the mills.

The wheat samples were ground fine enough to pass through a 1 mm. sieve. Immediately after grinding, the samples were extracted in duplicate with petroleum ether (b. p. 30–60° C.) according to the procedure recommended by Coleman (1929) for determination of the free acid in wheat oil.

The iodine numbers were run by the Hanus Method (A. O. A. C., 1925).

The acid numbers were determined by Coleman's (1929) procedure.

Results of the determinations of the iodine and acid numbers of the oils from the 1929, 1930, and 1931 wheat crops appear in Tables II and III.

Discussion

From an examination of Tables II and III, it will be noted that the iodine numbers of the wheat oils were very uniform. The average iodine number of the oils from the 1929 wheat crop was slightly lower than those for 1930 and 1931.

The acid numbers showed some variation. The oils from the wheats grown in Michigan had lower acid numbers than did those of the other states.

Effect of Storage of Ground Wheat on the Acid and Iodine Numbers of Wheat Oil

The samples of the 1931 wheat crop were ground and stored in mason jars for about 2½ months before the author found time to determine the iodine and acid numbers. The first 24 samples analyzed gave results which were very different from those obtained in previous years. Therefore, new samples of wheat were ground, a few at a time, and the iodine and acid numbers determined immediately. These results agreed very well with those of 1929 and 1930. In the case of unground wheat, there is apparently very little change in the oil con-

TABLE II
WHEAT OIL CONSTANTS OF WHEAT SAMPLES RECEIVED FROM MILLS

State	Mill	Iodine number			Acid number		
		Crop year			Crop year		
		1929	1930	1931	1929	1930	1931
District of Columbia	1	114.96	116.80	117.36	12.76	11.07	12.90
		117.04			11.58		
Georgia	1	114.13	116.65		9.65	13.69	
Indiana	1	116.27	115.64	117.31	10.50	13.02	13.07
Indiana	2		117.73	116.78		15.85	9.57
Indiana	3		116.73	116.75		13.93	12.36
Indiana	4	114.10	116.13		12.66	13.12	
Indiana	5		116.54			13.75	
Indiana	6	114.80	115.25	119.18	10.74	13.62	9.16
Illinois	1	114.79	117.48	116.57	10.71	16.15	8.11
Illinois	2		117.92	117.87		13.06	8.70
Illinois	3		116.39	119.75		13.21	8.96
Illinois	4	115.72	118.02	118.55	10.37	12.08	9.72
Kentucky	1	115.07	116.30	117.33	9.97	12.07	10.69
Kentucky	2	117.29	116.90	118.03	12.25	13.45	10.73
Kentucky	3	115.20	116.87		11.12	12.48	
Kentucky	4	115.91	117.48	116.81	11.06	12.42	8.93
Kentucky	5	114.21			10.65		
Michigan	1		118.73	116.87		8.50	8.45
Michigan	2	115.27	117.00	119.62	7.29	8.94	6.72
Michigan	3	113.70	116.50	116.56	6.28	8.67	8.45
Michigan	4		116.76	116.40		8.40	9.12
Michigan	5		117.43	115.39		9.67	8.13
Michigan	6			116.85			8.70
Michigan	7	116.50			8.67		
Michigan	8	116.00			16.48		
Missouri	1	114.16	115.26	116.10	10.78	16.04	8.80
Missouri	2	114.60	118.25	115.48	11.81	10.21	8.85
Missouri	3	114.64	115.03	118.68	10.20	10.47	13.42
North Carolina	1		117.60	116.93		12.22	11.12
North Carolina	2		118.02			12.18	
Ohio	1	114.18	117.79	115.92	12.88	18.16	10.97
Ohio	2		116.07	116.07		13.82	10.78
Ohio	3	115.77	116.23		13.20	10.06	
Ohio	4			117.44			9.77
Tennessee	1	115.78	117.08	114.19	10.94	23.25	9.51
Tennessee	2		117.71	117.00		11.16	8.51
Tennessee	3	115.06	116.59	115.82	11.14	14.33	9.31
Tennessee	4	114.50			11.76		
Virginia	1		117.38	116.71		12.08	8.67
West Virginia	1			115.62			11.50
West Virginia	2			116.56			12.82
Average		115.19	116.91	116.98	11.01	12.76	9.88
High		117.29	118.73	119.75	16.48	23.25	13.42
Low		113.70	115.03	114.19	6.28	8.40	8.11

stants upon storage. On the other hand, the iodine number decreases and the acid number of the oil increases very rapidly when ground wheat is stored. This fact may be of interest to millers of graham or whole wheat flour. The results of this storage experiment are recorded in Table IV.

TABLE III

WHEAT OIL CONSTANTS OF PURE VARIETY WHEAT SAMPLES RECEIVED FROM AGRICULTURAL EXPERIMENT STATIONS

Agricultural Experiment Station	Wheat variety	Iodine number		Acid number	
		Crop year		Crop year	
		1930	1931	1930	1931
Indiana	Fultz	118.50	116.14	8.50	7.36
Indiana	Michigan Amber	117.72	114.10	8.76	8.49
Indiana	Purkoff	116.58	114.00	7.68	8.08
Indiana	Red Cross	117.11	116.37	9.05	8.57
Indiana	Rudy	117.27	115.43	10.70	7.13
Indiana	Trumbull	118.36	115.40	11.16	8.53
Illinois	Fulcaster	117.06	116.68	7.64	11.74
Illinois	Fulhio	118.30	116.71	8.82	11.93
Illinois	Fultz	117.83		6.72	
Illinois	Illinois Chief	118.56		7.53	
Illinois	Illinois Progress No. 2	117.50	116.88	8.62	13.45
Illinois	Michigan Amber	117.65	117.61	6.28	12.88
Illinois	Nabob	116.82		7.56	
Illinois	Poole	116.18		7.30	
Illinois	Purkoff	117.73	117.23	7.66	10.40
Illinois	Red Cross	116.71	117.92	8.58	8.33
Illinois	Red Sea	117.00		8.50	
Illinois	Shepherd	117.73		6.10	
Illinois	Winter Fife	115.43		5.88	
Illinois	May		117.54		12.43
Michigan	American Banner	118.35	118.92	6.58	8.75
Michigan	Bald Rock 0814	116.23	116.84	6.01	8.53
Michigan	Bald Rock 0815	116.76		5.76	
Michigan	Red Rock		117.81		5.12
Michigan	Selection 912203		117.95		10.16
Missouri	Fulcaster	116.66		7.05	
Missouri	Fulhio	117.30		8.20	
Missouri	Harvest Queen	117.25		8.97	
Missouri	May	117.83		8.55	
Missouri	Michigan Wonder	116.04		6.30	
Missouri	Poole	115.15		7.21	
Ohio	Dawson's Golden Chaff	118.54	119.24	11.95	11.13
Ohio	Fulcaster	117.67	118.72	14.28	11.63
Ohio	Fulhio	118.28	118.89	14.17	16.40
Ohio	Gipsy	118.19	115.57	13.58	14.30
Ohio	Gladden	115.74	113.54	14.87	13.04
Ohio	Goens	116.80	117.57	12.87	12.29
Ohio	Mediterranean	116.20	116.41	13.15	18.57
Ohio	Nabob	115.16	115.11	11.72	13.15
Ohio	Nigger	119.07	117.76	13.23	17.08
Ohio	Nittany	116.75	117.36	13.84	13.42
Ohio	Poole	116.78	116.40	13.02	14.20
Ohio	Red Wave	115.47	115.91	16.36	13.98
Ohio	Rudy	116.24	117.54	13.47	17.36
Ohio	Trumbull	118.03	116.97	15.32	14.08
Pennsylvania	Forward	116.10	114.56	7.52	11.90
Pennsylvania	Fultz	117.35	116.34	7.15	12.18
Pennsylvania	Nittany (Pa. 44)	116.08	115.94	7.64	13.42
Pennsylvania	Fulcaster		116.58		12.13
Virginia	V. P. T. No. 131	116.90		13.63	
	Average	117.10	116.68	9.68	11.77
	High	119.07	119.24	16.36	18.57
	Low	115.15	114.00	5.76	5.12

TABLE IV
SHOWING THE EFFECT OF STORAGE OF GROUND WHEAT ON THE IODINE AND THE
ACID NUMBERS

Sample	Iodine number		Acid number	
	Original	Stored	Original	Stored
1	117.31	114.70	13.07	51.91
2	116.75	110.65	12.36	63.28
3	119.18	114.26	9.16	38.30
4	116.78	112.10	9.57	43.32
5	116.57	111.92	8.11	28.22
6	118.55	114.40	9.72	37.61
7	117.87	112.29	8.70	35.80
8	119.75	114.57	8.96	30.58
9	115.92	111.86	10.97	56.76
10	116.07	111.57	10.78	60.91
11	117.44	109.50	9.77	43.57
12	115.48	117.36	8.85	28.33
13	118.68	114.96	13.42	60.66
14	116.10	109.91	8.80	34.16
15	117.33	110.05	10.69	54.12
16	118.03	111.15	10.73	55.93
17	116.81	110.54	8.93	39.79
18	116.87	114.55	8.45	32.70
19	116.40	113.74	9.12	50.97
20	116.56	114.35	8.45	36.86
21	115.39	111.80	8.13	34.97
22	119.62	117.90	6.72	31.21
23	116.85	115.42	8.70	38.24
24	114.19	110.38	9.51	42.80

Discussion

The iodine numbers of the wheat oils decreased 2 to 8 points when the ground wheat was stored for $2\frac{1}{2}$ months. During the same period, the acid numbers of the wheat oils increased 2 to 6 fold.

Summary

1. The iodine numbers of wheat oils were quite uniform from year to year during the period tested regardless of the source of the wheat.
2. The samples of pure varieties of wheat had approximately the same iodine numbers as the wheat received from the mills.
3. The iodine number of the oil of ground wheat decreased and the acid number increased during storage.
4. The oils from the Michigan wheats had the lowest acid numbers.
5. From the results obtained, it appears that a determination of the iodine number would be of no value in predicting the relative susceptibility of different wheats to the development of rancidity.

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THE COMPARATIVE COMPOSITION OF BROWN AND POLISHED RICE—THE LOSSES IN MATERIAL DUE TO POLISHING

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The comparative composition of brown rice and polished rice is of peculiar interest just now, in view of the extensive research to determine their relative nutritional value.

Sometime ago five samples of brown rice and the corresponding samples of polished rice, all from the same lots, were obtained from Japan and analyzed.¹ The analytical data are to be found in Tables I and II. Besides these ten samples, a sample each of "brown" glutenous² rice and "polished" glutenous rice, obtained from Japan, was also analyzed. The findings are shown in Table III.

The analyses showed that glutenous rice is appreciably richer in fat and fiber and heavier than are the common rices, but it is not

TABLE I
COMPOSITION OF BROWN RICE

Sample number	Moisture	On moisture free basis					
		Fat	Crude fiber	Ash	Protein (Nx 6.25)	Carbo-hydrates	Weight per 1000 kernels
	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	Gms.
1	9.44	2.50	0.98	1.38	8.69	86.45	17.94
2	9.22	2.17	.92	1.12	9.06	86.73	20.90
3	9.48	2.52	.85	1.22	7.69	87.72	20.93
4	8.97	2.57	.86	1.19	10.25	86.13	16.94
5	9.20	2.49	.80	1.18	7.69	87.84	23.92
Average	9.26	2.45	.88	1.22	8.67	86.70	20.11

¹ The analytical work was done by R. M. Bohn, formerly Assistant Chemist.

² The term "glutenous" which suggests gluten or protein and which implies that the product is rich in nitrogenous compounds is rather an unfortunate one. That it has no reference to the protein or gluten content is well known. The term is simply suggestive of the fact that when boiled, glutenous rice becomes more gelatinous and sticky than do the common rices. The chief use of glutenous rice is in making ame, midzu ame, and other Japanese confections. This rice is not on the American market to any appreciable extent.

TABLE II
COMPOSITION OF POLISHED RICE

Sample number	Moisture	On moisture free basis					
		Fat	Crude fiber	Ash	Protein (Nx 6.25)	Carbo-hydrates	Weight per 1000 kernels
	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	Gms.
1	9.63	0.25	0.15	0.31	8.25	91.04	16.63
2	9.18	.36	.18	.37	8.31	90.88	19.10
3	9.62	.45	.15	.41	7.19	91.80	18.76
4	9.02	.26	.14	.35	9.56	89.69	14.57
5	9.42	.55	.17	.35	7.44	91.49	22.12
Average	9.37	.37	.16	.36	8.15	90.79	18.24

TABLE III
COMPOSITION OF GLUTENOUS RICE

Sample	Moisture	On moisture free basis					
		Fat	Crude fiber	Ash	Protein (Nx 6.25)	Carbo-hydrates	Weight per 1000 kernels
	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	Gms.
Brown	9.08	2.89	1.12	1.36	8.63	86.00	26.5
Polished	9.37	.56	.28	.41	8.75	90.00	23.0

TABLE IV
LOSS IN WEIGHT AND IN CONSTITUENTS OF RICE DUE TO POLISHING

Sample number	On moisture free basis					
	Fat	Crude fiber	Ash	Protein (Nx 6.25)	Carbo-hydrates	Weight per 1000 kernels
	P.ct.	P.ct.	P.ct.	P.ct.	P.ct.	Gms.
<i>Common rice</i>						
1	90.8	85.8	79.6	12.0	4.3	7.3
2	84.8	82.1	69.8	16.1	4.2	8.6
3	83.9	84.1	70.0	16.2	6.2	10.4
4	91.3	86.1	74.7	19.7	10.1	14.0
5	79.6	80.4	72.5	10.4	3.8	7.5
Average	86.1	83.7	73.2	14.9	5.7	9.5
<i>Glutenous rice</i>						
	83.2	78.3	73.3	12.2	9.2	13.2

always richer in protein. Three samples of the common rices contained more protein than was found in the glutenous rice (Tables I and III).

From an examination of Tables IV and V it can be shown that in

TABLE V
COMPOSITION OF ROUGH RICE (RICE IN THE HULL) ¹

	Minimum	Maximum	Average
Weight per 1000 kernels (Gms.)	27.82	32.50	29.29
Moisture (P.ct.)	9.10	11.52	10.28
Protein (P.ct.)	6.83	8.40	7.95
Fat (P.ct.)	1.44	2.04	1.65
Fiber (P.ct.)	9.45	11.44	10.48
Ash (P.ct.)	3.26	4.66	4.09
Carbohydrates (P.ct.)	63.01	65.70	65.60

¹ U. S. Bureau of Chemistry Bull. 45.

producing approximately one billion pounds of polished rice (the average annual yield in the United States), the material removed by polishing brown rice is equivalent to one hundred million pounds. This contains approximately twenty million pounds of fat, twelve and one-half million pounds of protein, and nine million pounds of mineral ingredients. Practically all this material is disposed of as feed.

The importance of mineral ingredients for human nutrition is being recognized more and more. According to other analyses, not here recorded, more than 50% of the ash of rice is a combination of phosphorus with calcium, potassium, magnesium, and sodium; fully 25% of it being in the form of potassium phosphate. During the process of polishing the rice crop of the United States each year, approximately five million pounds of phosphorus and two million pounds of potassium compounds are removed. The polishing of rice, therefore, causes large losses in mineral elements which are useful to man. Also, as is well known, this process removes the so-called aleurone layer (or sub-pericarpel layer) which is especially rich in protein and vitamins, as well as in minerals.

In this country polished rice is generally regarded as having keeping qualities superior to those of brown rice, but in Japan, when it is necessary to carry rice over from one season to another, brown rice and not polished rice is stored. The samples of brown rice used in this investigation were still in good condition after having been kept in the laboratory in small bottles at room temperature for more than two years. The keeping qualities of food products of this kind moreover, can be enhanced by proper storage in a cool, dry place.

SOME FACTORS WHICH AFFECT GAS PRODUCTION DURING DOUGH FERMENTATION

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(Read at the Convention, May, 1932)

Introduction

The subject of gas production during dough fermentation has been studied by chemists for the past quarter of a century or more, and is not a new one in any sense. Bailey and Weigley (1922), Bailey and Johnson (1924), Richards (1925), and Fisher and Halton (1929) have all contributed something to the subject.

Wheat from certain localities in the Southwest during the past two years seemed to lack diastatic activity. This was evidenced by the crust color of the baked loaves which were, in some cases, pale and mottled. However, the use of the baking test as a measure of diastatic value with its many uncontrolled factors is often misleading. The possibility of the gas test being used to measure or evaluate the gassing power, and thereby serve as an index to the diastatic activity of the flour, was thought desirable.

Experimental

Apparatus and Material

The work reported in this paper was started in February, 1931 and covers flours milled from the 1930 and 1931 wheat crops. Before carrying on investigations on this subject, it was necessary, of course, to develop suitable apparatus capable of making duplicate gas checks on comparatively small amounts of dough. After trying a number of apparatus, one was adopted very similar to that described by Kent-Jones (1927). The apparatus is very simple and can be easily constructed in any cereal laboratory. It is illustrated in Figures 1, 2, and 3. Instead of using a salt solution of rather high density in which carbon dioxide is insoluble, a one-half inch layer of kerosene on distilled water, as proposed by James and Huber (1928), was used. The apparatus was carefully checked before use to see that the system was free from leaks and tests were made to determine whether replicated tests would agree.

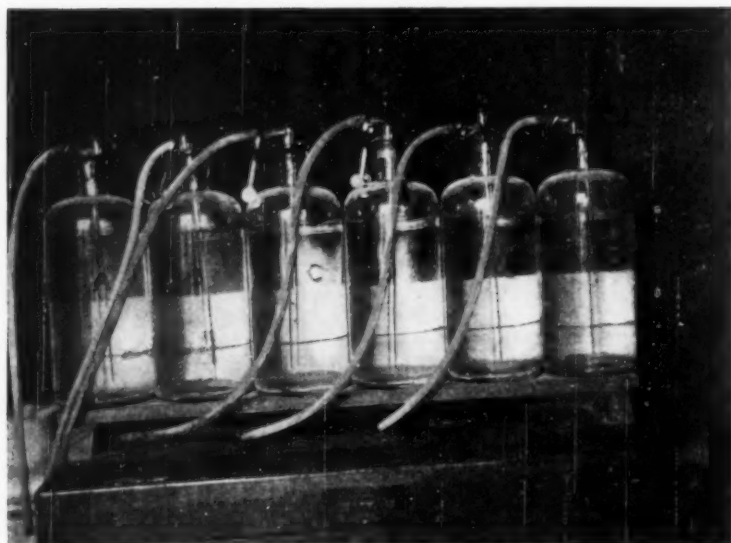


Fig. 1. Battery of gas collection apparatus.

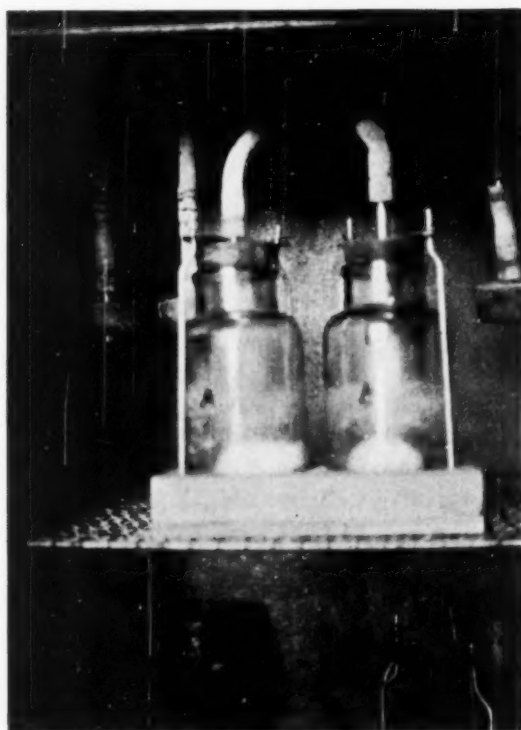


Fig. 2. Fermenting bottles showing clamp to prevent stoppers from slipping.

Before any standards for gas production of flours could be set up, it was deemed advisable to make a study of the factors which affect gas production. A search of the literature did not produce any data that could be used. In view of this fact it was decided to study some of these factors and it is the effect of these factors upon gas production that constitutes the major portion of this paper.

One of the first things necessary, of course, was to decide upon a standard formula. The standard formula adopted was as follows:

Flour	100 gms. (15% moisture basis)
Yeast	1.25 gms.
Milk	2.49 gms.
Salt	1.86 gms.
Sugar	2.48 gms.
Absorption	65%

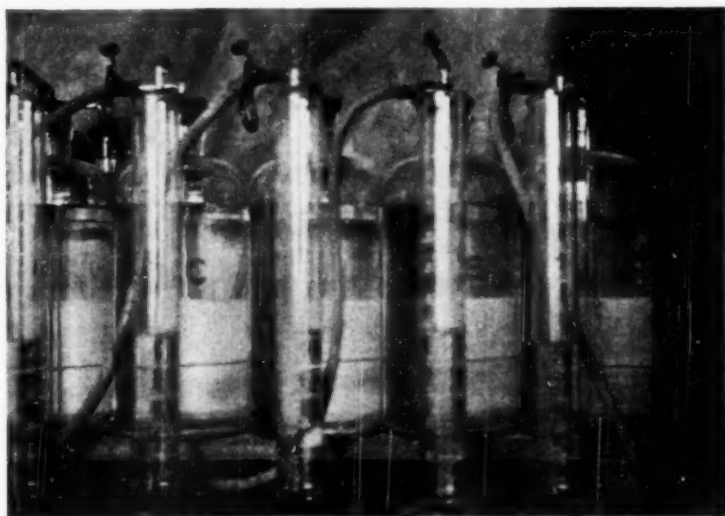


Fig. 3. Gas collection apparatus in operation.

Using the standard formula, tests were run for a few days to see if the different units in the apparatus would check. During these preliminary tests great variations in the yeast were noted from day to day. In order to study the influence of the yeast supply, the following procedure was adopted: The standard formula, as given above, was used each day and when the whole series of determinations was complete the average amount of gas produced with the standard formula was obtained and this average used to determine the data shown under the caption "Corrected displacement" in the tables. In no case are data reported on a single determination. The determinations were all made

in duplicate and if the duplicates did not agree within 20 cc. over the six-hour period, they were repeated.

Method of Procedure

Frequent moisture tests were made on the flour in order that the correct amount would be used for each determination. Standard solutions of salt, sugar, and milk were prepared; and when used were accurately pipetted out. One hundred grams of flour, on a 15% moisture basis, were weighed out on a Torsion balance, and together with the other ingredients mixed in a Hobart Mixer with the Swanson attachment (Figure 4), running at a speed of 109 r.p.m., for one minute. This

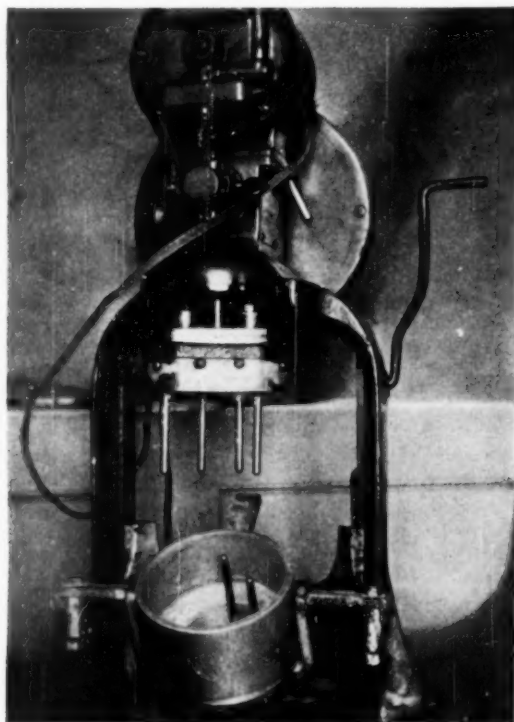


Fig. 4. Hobart mixer with Swanson attachment.

mixing time was adhered to in all cases except where the effect of mixing time was studied. The mixed dough was immediately scaled in duplicate at 70 gms. and placed in fermenting bottles (Figure 2). All doughs were brought out of the mixer as near 80° F. as possible. No dough should be used that varies $\pm 2^\circ$ F. from 80° F. As soon as possible the doughs were placed in bottles with a specially constructed

clamp to prevent the stoppers from slipping. The clamp and bottles were then placed in a Sargeant incubator adjusted to 80° F. (The fermenting bottles and clamp are always stored in the incubator so that a uniform temperature of 80° F. is maintained.) Five minutes time was allowed for the doughs to come to equilibrium, after which the water level was adjusted by blowing into the pressure outlet so that the water was just ready to drop. 100 cc. standardized graduates were placed under the overflow tube. The time was noted and a reading made every 30 minutes during the six-hour period. All of the total gas production curves represent the amount of water displaced.

The first flour studied was of the 1930 wheat crop, straight grade, of the following analysis: Moisture, 10.4%; ash, 0.53%; and protein, 10.9%. The flour carried a light bleach.

Influence of Quantity of Yeast on Gas Production

It was decided first to study the influence of varying percentage of yeast on gas production. The data are given in Table I. In this table,

TABLE I
QUANTITY OF YEAST VERSUS GAS PRODUCTION

Standard flour	Yeast	Water displacement	Corrected displacement ¹
Cc.	P.ct.	Cc.	Cc.
490	1.00	427	341
404	1.25	404	404
475	1.50	516	445
438	1.75	517	483
410	2.00	530	524
422	2.50	593	575
444	3.00	664	624

¹ Example: Table I, item 1. Standard, 490 cc., yeast 1%. Average of all standards in series of tests (Tables I, II, and III), 404 cc. Difference, 86 cc. Water displacement, 427 cc. Displacement minus difference equals 341 cc.—Corrected displacement.

the first column is designated as "Standard." Under this heading is listed the total gas production on each day when using the standard formula. With this particular flour there is a variation of 86 cc. in gas development between the high and the low. The average of all the standard tests made on this flour was 404 cc. The third column in Table I is labelled cubic centimeters displaced. This is the actual number of cubic centimeters of gas obtained with the different percentages of yeast. The fourth column "Corrected displacement" represents the number of cubic centimeters of gas after the addition or subtraction based on the standard for that day whether above or below the average for the completed test. In Figure 5 the data have been graphed. It will be noticed that the curve is practically a straight line.

With increasing amounts of yeast, the total amount of gas increases. This study turned out as expected; by increasing the yeast, the total amount of gas developed increased.

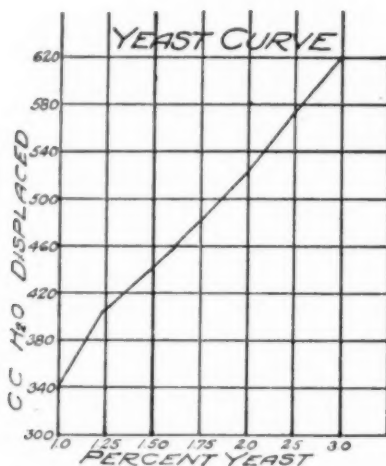


Fig. 5. Influence of quantity of yeast on gas production.

Influence of Salt on Gas Production

The next series of tests run was made varying the percentage of salt from 0 to 3.0%. These results are shown in Table II and Figure 6. Salt very materially alters the gas production of the dough. With each addition of salt the total gas production was less. The curve for increasing percentages of salt is practically the reverse of the yeast curve.

TABLE II
SALT VERSUS GAS PRODUCTION

Standard flour	Salt	Water displacement	Corrected displacement
Cc.	P.ct.	Cc.	Cc.
393	.0	490	501
428	.5	487	463
405	1.0	453	452
350	1.5	390	444
404	2.0	404	404
349	2.5	298	353
423	3.0	300	281

Influence of Sugar on Gas Production

The next series of tests related to the percentage of sugar on gas production. The sugar was varied from 0 to 5.0%. These results are shown in Figure 7 and Table III. The curve is very nearly a straight

line curve paralld to the horizontal axis, due in all probability to the rather low percentage of yeast used in the standard formula. The flour apparently was high enough in diastase to supply the yeast with sugar, since more added sugar did not materially increase gas production.

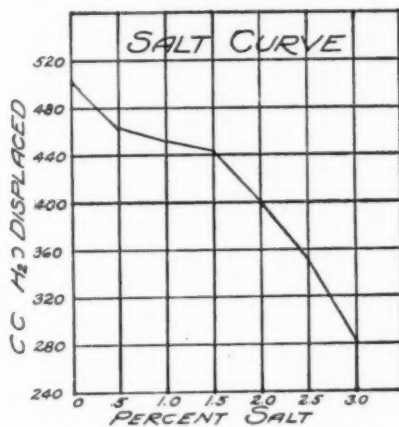


Fig. 6. Influence of salt on gas production.

TABLE III
SUGAR VERSUS GAS PRODUCTION

Standard flour	Sugar	Water displacement	Corrected displacement
Cc.	P.ct.	Cc.	Cc.
358	0.0	338	386
392	.5	388	400
380	1.0	370	394
390	1.5	385	399
388	2.0	377	393
404	2.5	404	404
362	3.0	373	415
386	3.5	392	410
392	4.0	388	400
422	5.0	403	385

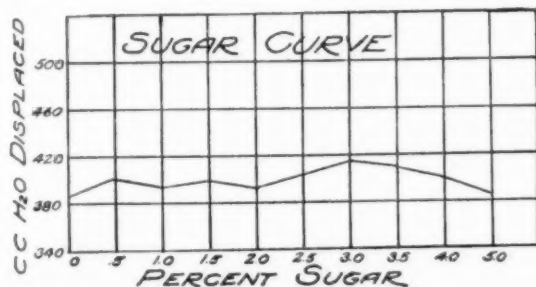


Fig. 7. Influence of sugar on gas production.

Influence of Absorption on Gas Production

The influence of water absorption was next studied. This was varied from 58% to 70%. The results of these tests are shown by the absorption curve, Figure 8, and in Table IV. In this connection a new standard flour of the 1931 crop was used in this and remaining tests

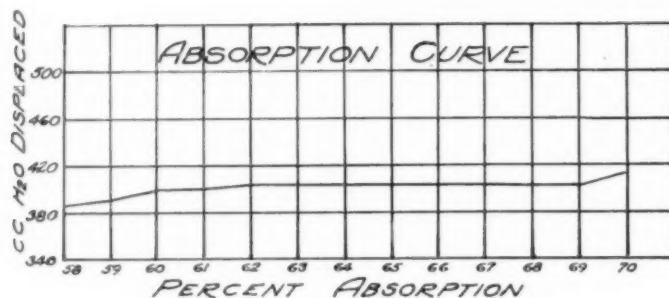


Fig. 8. Influence of absorption on gas production.

TABLE IV
ABSORPTION VERSUS GAS PRODUCTION

Absorption (P.ct.)	58	59	60	61	62	63	64	65	66	67	68	69	70
Standard flour (Cc.)	402	387	387	395	395	446	446	415	427	427	420	420	402
Water displacement (Cc.)	384	376	384	392	395	446	447	415	427	428	418	419	413
Corrected displacement	384	391	399	399	402	402	403	402	402	403	400	401	413

(Tables IV, V, VI, VII and VIII). A gradual increase in gas production from an absorption of 58% to 62% will be noticed in Figure 8. These doughs are stiffer than would usually be worked, and in most cases would be termed stiff. From 62% to 69% the curve is practically parallel to the horizontal axis. The doughs from 62% to 65% were about the right absorption. From 65% to 69% they were rather slack and sticky. The 70% dough was very slack and entirely too sticky to handle. On the absorption curve between 62% and 69% there is practically no difference in the total gas produced. Doughs that were very stiff produced slightly less gas. Doughs which were very slack produced slightly more gas than those of the proper consistency. From the data presented here it would be very safe to assume that absorption does not materially affect gas production since bakers would not work doughs at either of these extremes.

Influence of Yeast Food on Gas Production

Since Arkady is perhaps the most widely used yeast food, it was thought advisable to run a series of doughs using different amounts of Arkady. The Arkady curve, Figure 9, and Table V give the results of these tests. From 0.25% to 1.5% of Arkady were used. The

curve rises very rapidly to 0.75% Arkady. Amounts greater than 0.75% seemed to have a detrimental rather than a beneficial effect as regards gas production where 1.25% yeast was used. Had the formula contained a greater percentage of yeast no doubt a greater percentage of Arkady could have been used.

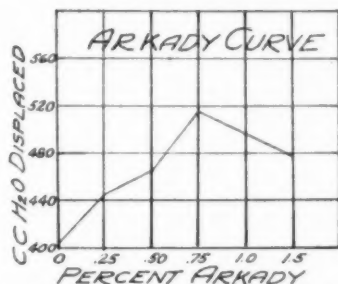


Fig. 9. Influence of yeast food (Arkady) on gas production.

TABLE V
ARKADY AND GAS PRODUCTION

Standard flour	Arkady	Water displacement	Corrected displacement
Cc.	P.ct.	Cc.	Cc.
387	0.25	432	447
387	.50	450	465
403	.75	517	516
403	1.00	497	496
415	1.50	491	478

Diastatic and Non-Diastatic Malts

The next investigation was relative to the use of diastatic and non-diastatic malts. It was found, as shown by the diastatic and non-diastatic curves in Figure 10, and from the data in Table VI, that the

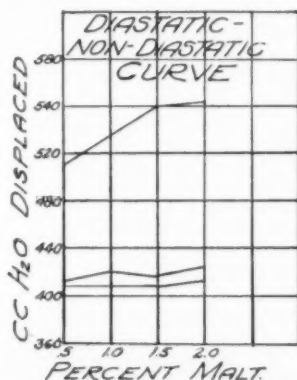


Fig. 10. Influence of malt on gas production.

addition of either the diastatic or non-diastatic did not materially change the amount of gas produced. This was to be expected since it was found that the sugar curve was practically a straight line.

The standard formula was changed so that it would contain 2% yeast and a series was run using only diastatic malt. These results are also shown in Figure 10, and the data given in Table VI. From the above results it seems logical to state that in the standard formula the yeast in no case was subjected to starvation. Jorgensen (1931) also gives a method of studying a baking formula to see whether the yeast starves.

TABLE VI
DIASTATIC AND NON-DIASTATIC MALT VERSUS GAS PRODUCTION

	<i>Non-Diastatic</i>			
Malt (P.ct.)	0.5	1.0	1.5	2.0
Standard flour (Cc.)	421	421	424	424
Water displacement (Cc.)	425	428	430	435
Corrected displacement (Cc.)	406	409	408	413
	<i>Diastatic</i>			
Malt (P.ct.)	0.5	1.0	1.5	2.0
Standard flour (Cc.)	398	398	403	403
Water displacement (Cc.)	406	417	420	425
Corrected displacement (Cc.)	410	421	419	424
	<i>Diastatic malt plus 2% yeast</i>			
Malt (P.ct.)	0.5	1.0	1.5	2.0
Standard flour (Cc.)	438	438	433	433
Water displacement (Cc.)	546	572	571	576
Corrected displacement (Cc.)	510	536	540	545

The studies made so far have had to do with changes in chemical composition of the formula as they influence gas production. It was likewise thought advisable to study the influence that physical changes might have on gas production. The effect of high speed mixing was first studied. The doughs were prepared for this test in the following way: The dough was mixed one-half minute on second speed (109 r.p.m.) to incorporate the ingredients, after which the mixer was operated at high speed (240 r.p.m.), the mixing lasting from 0.5 to 2.5 minutes.

In a former report Heald (1930), reporting on some experiments with high speed mixing using the Swanson modification of the Hobart mixer, found that the doughs began to break down after 2 minutes of mixing, as evidenced by the loaf volume and texture. The high speed mixing data are shown in Figure 11 and Table VII. From a gas production standpoint, the maximum was reached at 1.5 minutes. However, the maximum difference was only 30 cc. so that it can be safely said that within the range studied mixing time did not materially affect

the gas production. The only reason that a longer time was not used was because at 65% absorption and at 2.5 minutes of high speed mixing the dough was nearly a soup and entirely too sticky to handle.

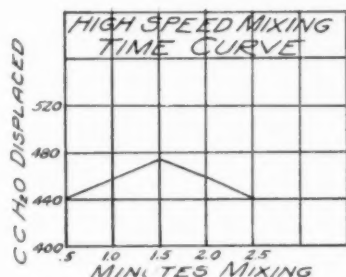


Fig. 11. Influence of mixing time on gas production.

TABLE VII
MIXING TIME VERSUS GAS PRODUCTION

Standard flour	Mixing time	Water displacement	Corrected displacement
Cc.	Minutes	Cc.	Cc.
424	0.5	447	440
424	1.0	465	458
405	1.5	458	470
440	2.0	489	462
475	2.5	499	441

Temperature and Gas Production

The last studies made had to do with the effect of elevated temperatures on gas production. A second incubator was used to run the standard each day as a check on the yeast supply. The doughs were taken out of the mixer as near the temperature studied as possible. The results of these tests are shown in Figure 12 and Table VIII.

It will be noticed in Figure 12 that at 80° F. the range through which a practically uniform amount of gas is given off is relatively great, being from 150 minutes to 300 minutes. At 85° F. the maximum amount of gas accumulated 30 minutes sooner than at 80° F., being at 210 minutes instead of at 240 minutes. At this temperature there was uniform gas production only 60 minutes. At 90° F. the peak comes 30 minutes sooner than at 85° F., i.e., at 180 minutes. The range of uniform gas production still further narrowed. At 95° F. the maximum gas production likewise takes place at 180 minutes. However, at this temperature gas production very rapidly declines during the next 30 minutes. The uniform gas production range is very sharp, being less than 30 minutes. At 100° F. we again find maximum gas

production at 180 minutes, but again a decided lessening in gas production takes place during the next 30 minutes.

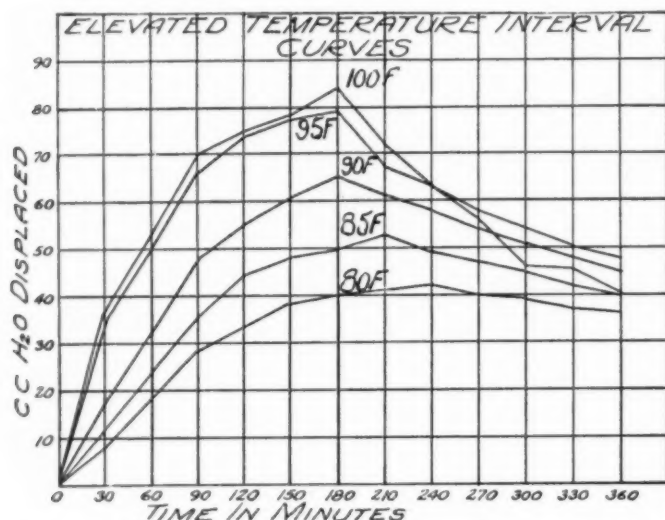


Fig. 12. Influence of temperature and time of fermentation on gas production.

TABLE VIII
TEMPERATURE VERSUS GAS PRODUCTION

Temper- ature	Time in minutes													Stand- ard flour	Total water dis- place- ment	Total cor- rected dis- place- ment
	30	60	90	120	150	180	210	240	270	300	330	360				
° F.														Cc.	Cc.	Cc.
80	8	18	28	33	38	40	41	42	40	39	37	36	402	402	402	
85	11	24	36	44	48	50	53	49	47	45	42	40	420	487	505	
90	18	32	48	55	61	65	61	58	54	51	48	45	398	596	600	
95	35	50	66	74	77	79	67	63	58	54	50	48	402	720	720	
100	36	53	70	75	78	84	72	63	56	46	46	40	402	718	718	

From the elevated temperature total gas production curve (Figure 13) we have practically a straight line curve of the same order as the yeast curve (Figure 1) up to 90° F., beyond which it breaks. These data would seem to prove that temperatures above 80° F. will increase the total gas production of a dough up to 95° F., beyond which there is a decline in the amount of total gas produced. Temperatures above 80° F. shorten the range through which uniform gas will be produced. Higher temperatures cause maximum gas production in a shorter time.

Summary

A method has been described which will check duplicate gas determinations on relatively small doughs during fermentation.

From the data presented in this paper, the factors which increase gas production in dough are: (a) Increased percentages of yeast; (b) sugar and diastatic malts when the diastase in flour does not furnish enough sugar for yeast growth; (c) yeast foods (Arkady) up to certain amounts depending on percentage yeast in formula; and (d) elevated temperatures up to 95° F.

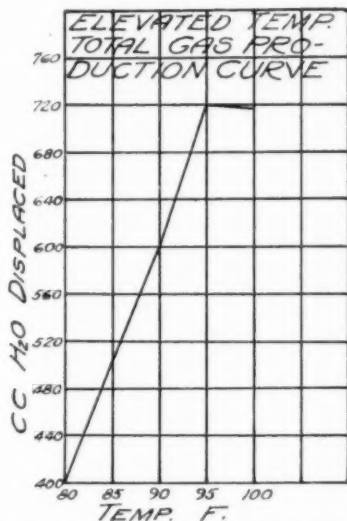


Fig. 13. Influence of high fermentation temperatures on gas production.

The factors which decrease gas production in doughs are: (a) Salt; (b) excessive amounts of yeast foods (Arkady); and (c) excessively elevated temperatures above 100° F.

The factors which apparently have no effect one way or another on gas production in doughs are: (a) Sugar and diastatic malts if flour contains sufficient diastase; (b) high speed mixing; and (c) absorption between the limits which would be used in ordinary dough fermentation.

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STUDIES WITH THE FARINOGRAPH FOR PREDICTING THE MOST SUITABLE TYPES OF AMERICAN EXPORT WHEATS AND FLOURS FOR MIXING WITH EUROPEAN SOFT WHEATS AND FLOURS

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(Read at the Convention, May, 1932)

Introduction

The typical European flour is made of a mixture of domestic soft wheats and hard wheats from the North American Continent. The percentage of a hard wheat in the mixture is not uniform throughout Europe but depends on a number of factors. Before the War the trade flours of the large mills contained up to 70% and even 80% of imported hard wheats, the remainder consisting of hard wheats of domestic or South American types. Such high percentages, however, were to be found only in those mills which were located near harbors and canals; the smaller mills, not so favorably located, could not afford to use as large a percentage of North American wheats in their mixture and used but from 10% to 20% of hard wheat.

During the last few years, because of the economic depression, most European countries have limited by law the percentage of foreign wheats that can be added to the mill mixture. In Germany, at this writing, the percentage permitted is only 3%. However, there is a loophole in the law, which permits the addition of 30% of foreign wheats if an equal amount of domestic wheat is exported by or through the mills, and only upon this export-certificate have the mills permission to import foreign wheats.

When these laws became effective the mills naturally had great difficulties in making flours which would permit the baker to continue bread making in the former manner. It became necessary for the mills to use for their mixture only the very highest quality of wheat and it has generally been recognized that No. 1 Manitoba Northern would give the best results when mixed with domestic wheats. It is understood, of course, that there is not as much No. 1 Manitoba Northern available as is sold in Europe, and that similar wheats from surrounding Canadian provinces are also sold as No. 1 Manitoba Northern.

The mills soon discovered that the grade specifications for imported hard wheats from the North American Continent were not correlated with the baking value of such wheats. It was found that the addition of only 20% of *one* shipment of No. 1 Manitoba Northern brought a very decided improvement in flour quality, while such an improvement could not be obtained with the addition of as much as 30% of *another* shipment of No. 1 Manitoba Northern in spite of the fact that grade specifications and laboratory reports did not show decided differences between the two wheats.

In order to uncover the reasons for this strange behavior many types of flour made from wheats grown on the North American Continent have been studied. Before proceeding with the results of these investigations, it will be necessary, however, to show you a typical Farinograph curve and explain its meanings. See Figure 1.

Explanation of Farinograph Curve

Distance *a* represents *the consistency of the dough*, which will vary with different amounts of water. An average drawn from the consistencies most commonly worked with by bakers appears to be about 500 degrees in the Farinograph.

Distance *b* represents *the so-called dough developing time* which depends on the speed with which water is absorbed by the flour.

Amplitude *c* depends greatly on the elasticity of the dough, and

Distance *d* is the *length of time* during which this elasticity exists. The *elasticity* is taken to be a measure of the possible optimum volume and the distance *d* tells us in what manner the dough will withstand mechanical and fermentation abuse. In other words, it shows us the fermentation tolerance not including, of course, those factors which are necessary for proper gas-production.

Angle *e* shows us *the degree of drop of the curve* which again is a measure of the stability of the gluten against mechanical and chemical influences. In other words, it can be used as a summary of the factors *c* and *d*.

Distance *f* is of particular importance and will be referred to often throughout this paper.

Farinograph Studies with Various Flours

Figure 2 shows that there is a tremendous difference in the time which is needed to bring a soft wheat and a hard wheat flour to its optimum point of development. It can be seen that the soft wheat curve has reached the highest point of development after 4 minutes, while the hard wheat curve has reached that point after 12 minutes. It is apparent that two wheats with such extremely different character-

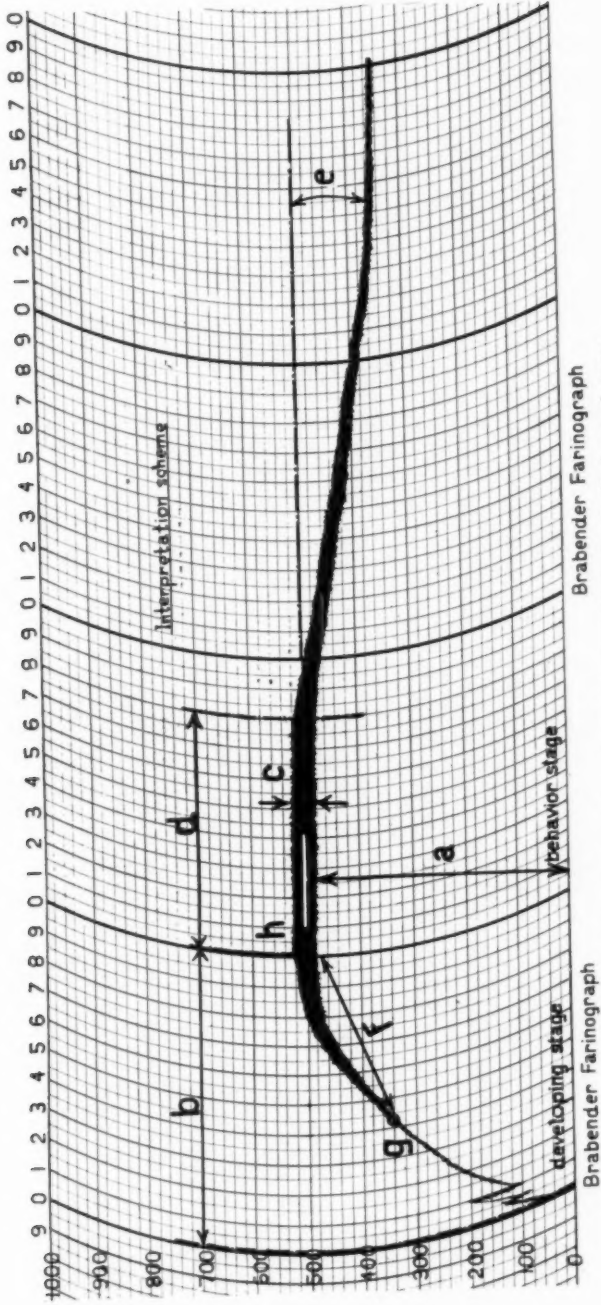


Fig. 1. Points of importance in interpreting Brabender Farinograph curve.

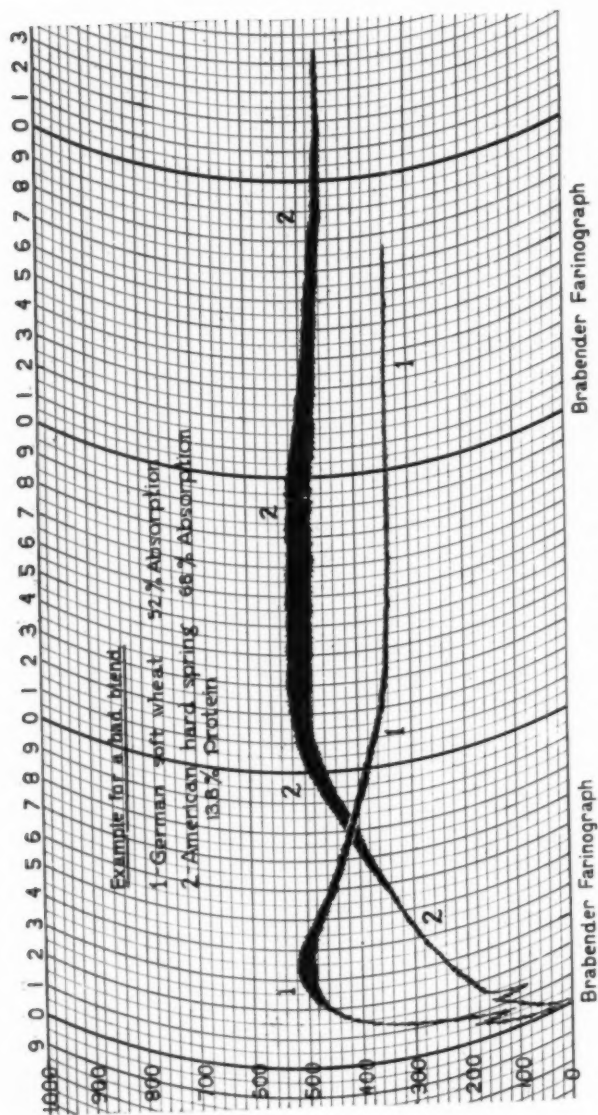


Fig. 2. Example of a bad blend, showing difference in time needed to bring a soft wheat and a hard wheat flour to optimum point of development.

istics will not harmonize if they are brought together in a mixture. Depending on the mode and time of mechanical development, either the soft wheat portion will be brought to its highest point of development when the hard wheat portion is still undeveloped, or the hard wheat portion will be given its optimum mixing and under such conditions the soft wheat flour will be greatly over-abused and its gluten killed. However, the characteristics of the hard wheat shown in this curve, are not necessarily typical of all hard wheats. There are other hard wheats whose speed of water absorption is much greater and whose highest point of development can be reached much earlier.

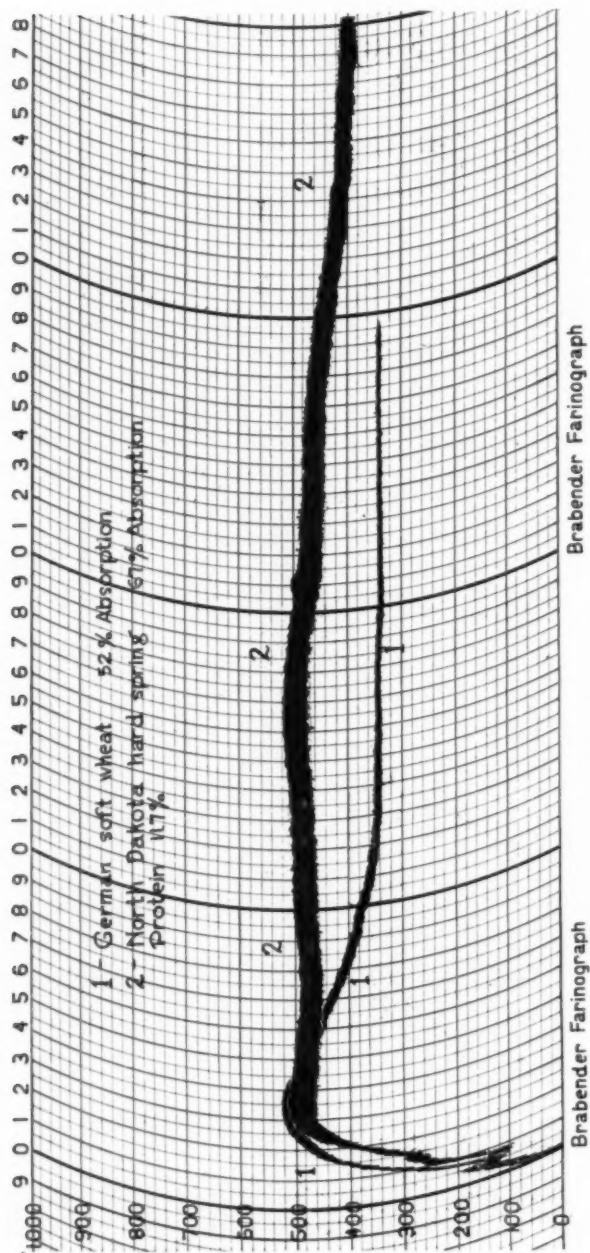
Figures 3 and 4 indicate the action of such hard wheats whose speed of water absorption is almost that of a soft wheat but whose ability to stand up under severe mechanical abuse is typical of a hard wheat. Some of the American flours from the Central Northwest and most of the Canadian flours show these characteristics. That similar characteristics can also be found in Kansas wheat can be seen from an examination of Figure 5.

As a matter of fact, most of the German millers have been pretty well satisfied with the Kansas wheat bought last year from the Farm Board as it gave good results in respect to loaf volume, grain, and texture. They noticed, however, that the sensitivity of these wheats to mechanical abuse was higher, while their fermentation tolerance was shorter than that of Northwestern and Canadian types. Proof of this was made with the Farinograph and it can be seen from the curves that the fermentation tolerance of these Kansas wheats—that is the standing up of the curve—as well as the amplitude, is actually less than with spring flours.

The wheats illustrated in Figures 3 to 5 appear ideally adapted for mixing with European soft wheats and to improve the baking value of those soft wheats to such a degree that ordinarily would be beyond all expectations.

It is not within the scope of this paper to give you the reasons for these phenomena but rather to describe to you the practical advantages that can be gained from flour studies by use of the Farinograph.

There is yet another factor to be considered by those who export wheat and flour to Europe. It is generally unknown among the trade that there are no high speed mixers used in Europe. Small bakers still make their doughs by hand while the large bakeries mix them in slow speed mixers of an entirely different construction than the American high speed mixer. Hard wheat doughs mixed in that manner have to develop mostly through the natural swelling effect while their *optimum* development can only be reached through the high speed effect of the American type of mixer which is capable of increasing the water ab-



Figs. 3 and 4. Examples of hard red spring wheat flours which will blend well with German soft wheats.

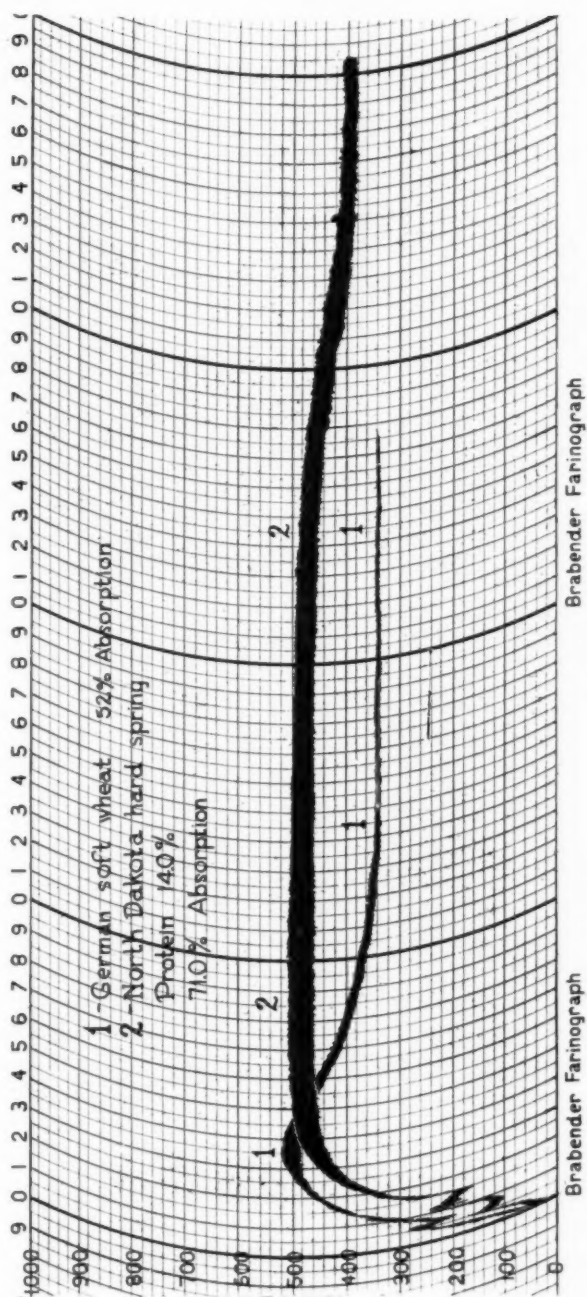


Fig. 4.

sorption capacity of most of the strong hard wheat flours of the North American Continent. In fact, such flours, if mixed in one of the slow speed European mixers will never reach a gluten development necessary to make a satisfactory loaf, in addition to the fact that their water absorption capacity remains at a much lower level when developed in such type of mixer. On the other hand, such flours need the high speed effect (mechanical modification) that the high speed mixer imparts to them. That it is impossible to reach the same goal with slow mixing and natural swelling is demonstrated by Figure 6, in which 3 curves, made from the same flour, are given. In each case 300 gms. of

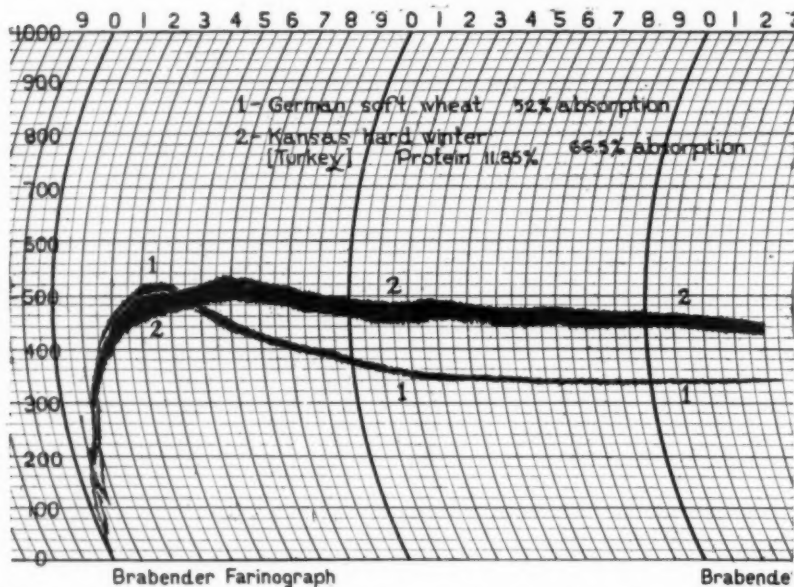


Fig. 5. Characteristic curve of Kansas wheat flour which will blend satisfactorily with German soft wheats.

flour were taken and 72% of water added. The dough represented by curve 2 was allowed to run its course; while with the dough represented by curve 3 a rest period of 45 minutes was introduced after $2\frac{3}{4}$ minutes of mixing. Further development of the curve, after 45 minutes, shows that the rest period was responsible for very little increase in the dough consistency; in other words, that it could not replace the dough development brought about by intensive high speed mixing (mechanical modification).

The same test was repeated a number of times, each time increasing the length of the rest period. The effect of longer rest periods was so small, however, that it is convincing proof that no appreciable development of strong American flours can be brought about through natural

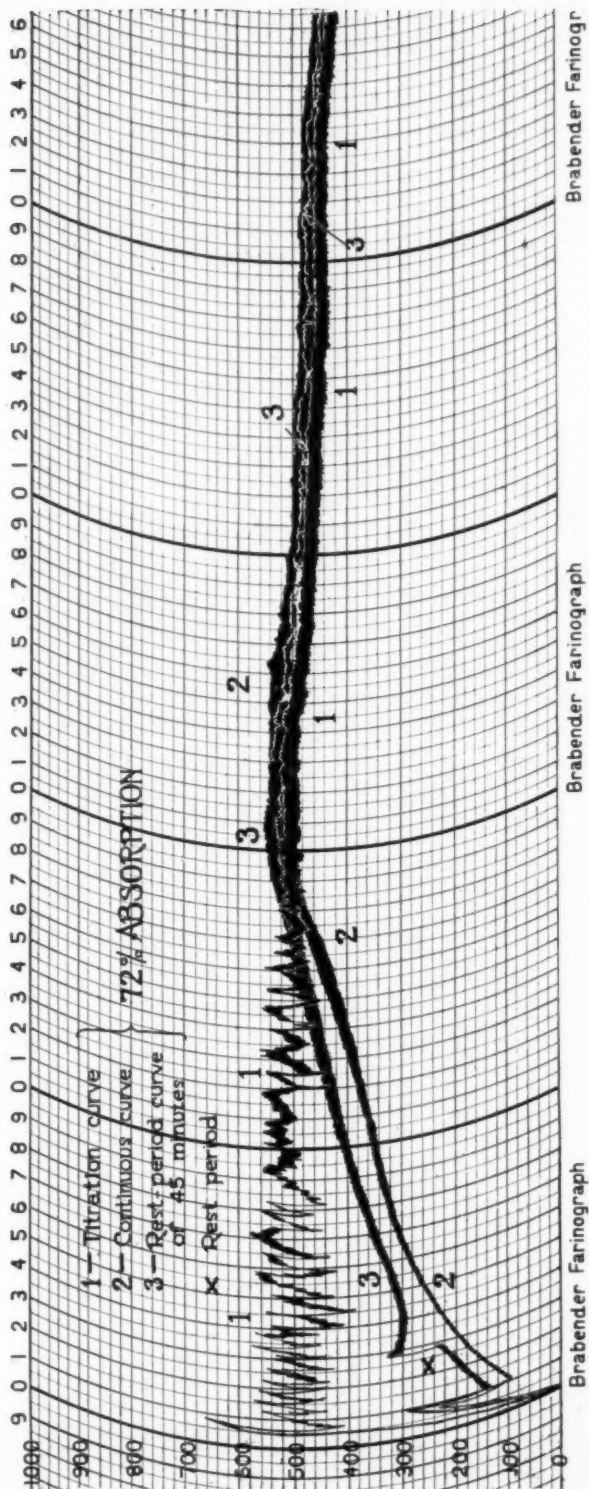


Fig. 6. Development of dough characteristics by high speed mixing.

swelling. That these factors, to a certain degree, were recognized long ago is probably the reason for the introduction of the high speed mixer in the American bakery practice. That their full significance has been known, however, is open to question.

With respect to the effect of the high speed mixer, Professor Mohs of Berlin, has developed a theory which he explains in his recent book "Mehlchemie," which runs about as follows:

"Before the wheat has ripened in the fields, the single cells of each grain contain a large amount of water. The regular climate of the North American Continent brings about a slow drying-out of the grain. With advancing ripening the drying-up process continues and the single cells in each grain get closer and closer to one another, until finally the ripe wheat has decreased its moisture content to as little as 11% or 12%. Through the evenness of the drying-out process the cell walls remain smooth and adhere to one another like two smooth pieces of glass, which, if put one upon the other can be separated only with difficulty.

"In Europe, on the other hand, the climate does not permit a regular ripening and drying-out of the grain. Rain and sunshine interchange in Europe much more often than on the North American Continent and as soon as the grain cells have dried out a little, rain sets in and the grain cells swell again. This continued alternate drying and swelling causes the formation of barky cell walls, somewhat like tree-bark, and these barky cell walls prevent the adhesion of the cell walls just like two rough glass plates will not adhere to one another."

When a dough is made from such domestic European soft wheat with rough cell surfaces, the water can easily penetrate between the cells and swell the dough relatively soon. With hard wheat the case is different. As the cell walls adhere to one another so strongly, water will only partially penetrate and swelling is greatly handicapped. The work of the high speed mixer which tears apart the single cells enables the water to penetrate and thus makes possible the proper development of strong American flours.

How tremendous the effect of the high speed mixer can be, is shown in the following experiment:

A pure Manitoba flour, milled by a large German mill, which was destined to be mixed with soft wheat flour, was returned to the mill because it would not sufficiently improve the soft wheat flour. The flour was baked in the test-bakery of the mill and produced a very poor loaf with a volume of only 1260 cc. A curve of the same flour was then made with the new high speed Farinograph, using the same formula as before. When baked, this last loaf showed a volume of 1915 cc. Cross sections of the two loaves are shown in Figure 7.

These results lead us to think and should arouse a great deal of curiosity among those who are interested in the export of wheats and flour to Europe. It is a fundamental error to think that the quality requirements for America are identical with the requirements made in Europe, and these new possibilities of determining whether a flour will fulfill the proper European requirements, should arouse great interest. We are fully convinced that many a good wheat has been condemned in Europe while this same wheat really has extraordinary qualities and would be more than satisfactory on the American market. On the

Effect of High Speed Mixing on an European Loaf Using Strong American Flour.

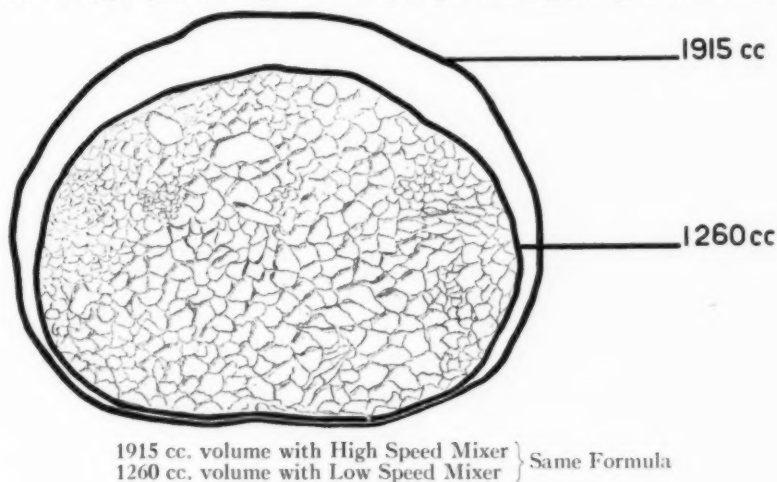


Fig. 7. Effect of high speed mixing on bread quality.

other hand, we are convinced that many a wheat and flour which gives poor results under American conditions, would fulfill the European requirements and be highly satisfactory in connection with European domestic wheats. In other words, wheats and flours should be selected for export to Europe which have a speedy water absorption capacity and fast developing time and also remain long on their optimum consistency. The American wheat and flour exporter, therefore, who desires to compete successfully on the European market, is advised to have flour samples come from Europe in order to be able to select wheats and flours for export whose characteristics are related to those of the European domestic wheats.

VARIATION IN THE WEIGHT OF A GIVEN VOLUME OF DIFFERENT FLOURS—III. CAUSES FOR VARIATION, MILLING, BLENDING, HANDLING, AND TIME OF STORAGE

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(Received for publication March 16, 1932)¹

In experiments described in a previous paper (Grewé, 1932a) considerable variation was found in the weight in a given volume of flour milled in the same section of the United States. Seven samples of flour milled in the State of Indiana in 1926 weighed as follows: 94.0, 91.2, 93.6, 92.6, 88.2, 92.5, and 87.7 gms. per cup. A more extreme case was found among flours milled in Illinois in 1928, in which four samples varied in weight from 82.1 gms. to 94.3 gms. When touched with the fingers the flour which weighed the less per cup had the feel of a finely ground flour.

Further experiments, presented in a second paper (Grewé, 1932b), showed that some of this variation may be due to the wheat used in making the flour.

In the present investigation, it was demonstrated that factors in milling and handling flour may also be causes of variation in weight. Degree of fineness and uniformity in size of flour particles are of considerable importance in baking quality. It is generally conceded that granulation of flour is a factor in determining the character of the cake and that in general a flour of fine granulation is preferred to one of coarse granulation. It is necessary that the miller give consideration to particle size in order to produce flour of maximum baking quality.

Flour is the basic ingredient in batters and doughs. As shown in the two studies previously mentioned, different flours of the same type may vary considerably in weight per unit of volume taken when the flour is measured by volume and not by weight, and such variations may have an important effect on the results of baking. On account of the importance of the ratio of flour to other ingredients, a study was made of some of the effects on weight per given volume of flour taken, due to the methods of milling, blending, storing, and handling the flour.

¹ Read at the Convention, May, 1930.

Method

The method followed was exactly the same as that in the previous study (Grewé, 1932a) except that a weighed amount (150 gms) of flour was used.

Effect of Size of Flour Granules

A sample of soft wheat flour was sifted on 13XX, 14XX, 15XX, and 16XX sieves. The portions remaining on these sieves varied in weight from 83.3 gms. per cup for the finest grained flour, to 95.7 gms. per cup for the coarsest grained flour (Table I). Evidently size of granule is a very important factor in weight per given volume of flour, and probably

TABLE I
EFFECT OF DIFFERENCE IN SIZE OF FLOUR GRANULES ON THE WEIGHT OF A CUP OF FLOUR

	Weight per cup
	Gms.
Original	87.6
Portion remaining on 13XX sieve	95.7
Portion remaining on 14XX sieve	89.6
Portion remaining on 15XX sieve	88.9
Portion remaining on 16XX sieve	86.8
Portion passing through 16XX sieve	83.3

is the cause of much of the variation encountered in samples of flour from different mills made from wheat grown in the same section. These results were confirmed by another set of samples which is not reported here.

Effect of Separating Flour Into Grades

Samples of the patent and the clear grade flours milled on five different days were examined for weight per cup (Table II). The average

TABLE II
EFFECT ON WEIGHT PER CUP WHEN FLOUR IS SEPARATED INTO GRADES. FIVE SAMPLES MILLED ON DIFFERENT DAYS

Patent	Clear
Gms.	Gms.
90.8	79.9
87.7	77.4
87.1	76.5
87.7	74.5
86.3	76.0

weight of the patent grade flour was about 11 gms. more per cup than that of the clear grade flour. This also confirms the findings of the effect of granule size on weight per cup of flour as the clear grade

flour was more finely ground than the patent. Similar results were secured on a similar series of flours from another mill.

Effect of Blending Flour

Five blends were prepared by using equal parts by weight of hard and soft wheat flour. It was found that the weight per cup of the blend was approximately the same as the average weight per cup of the flours used in making the blend (Table III). A number of commercial blends examined were found to be heavier than soft wheat flours and lighter than hard wheat flours.

TABLE III
EFFECT ON WEIGHT PER CUP OF FLOUR WHEN TWO FLOURS ARE BLENDED IN EQUAL PARTS BY WEIGHT

Weight per cup of the two flours used in making the blend		Average weight of the flours used in making the blend (calc.)	Weight per cup of the blend
Gms.	Gms.	Gms.	Gms.
87.8	116.6	102.2	102.4
89.5	110.9	100.2	99.5
87.8	114.9	101.5	101.4
91.1	114.9	103.0	102.9
90.8	114.9	102.8	102.4

Factors Introduced During Storage and Handling of Flour

That the moisture content of cereals and cereal products varies according to the temperature and humidity of the surrounding medium, has been known for many years and has been the subject of study by many investigators. As early as 1883, Brewer found that the amount of moisture in wheat depended upon the dryness of the air. Freshly milled flour has about 13.5% of moisture. When flour is held in heated rooms, it may lose as much as one-third of this moisture before it establishes an equilibrium with atmospheric humidity.

Effect of Moisture on the Weight of a Cup of Flour

Eight samples of fresh flour were received in tin containers sealed with paraffin. Upon arrival at the laboratory the samples were tested for moisture and weight per given volume, and the data are recorded in the first and second columns of Table IV. After the flours had been exposed for several days to the dry air of heated rooms in winter, they were again tested for weight per given volume and for moisture content. At this time, as shown in the third and fourth columns of this table, the flours had lost in moisture and gained in weight per given volume. These samples of flour were then transferred to paper bags and retained until the next summer. During a period of high relative humidity

they were again tested for weight per given volume and moisture content, with the results shown in the seventh and eighth columns of the table. This time the flour had gained in moisture and lost in weight. Age of flour and temperature and humidity of room were probable sources of error in this work. Similar findings were obtained on another set of samples which are not included in the report.

TABLE IV

EFFECT OF CHANGE IN MOISTURE CONTENT ON WEIGHT PER CUP OF FLOUR AT ORDINARY ROOM TEMPERATURES AND HUMIDITIES OF WINTER AND SUMMER

Fresh flour before exposure		After exposure to dry air of heated rooms in winter				After exposure to the relatively moist air in rooms in summer			
Weight per cup	Mois- ture	Weight per cup	Mois- ture	Gain in weight per cup	Loss in mois- ture	Weight per cup	Mois- ture	Loss in weight per cup	Gain in mois- ture
Gms.	P.ct.	Gms.	P.ct.	Gms.	P.ct.	Gms.	P.ct.	Gms.	P.ct.
102.2	13.3	109.7	9.0	7.5	4.3	102.6	12.3	7.1	3.3
91.1	13.6	97.1	9.0	6.0	4.6	89.8	13.0	7.3	4.0
95.4	13.8	103.2	8.8	7.8	5.0	94.5	13.0	7.7	4.2
89.6	13.7	97.2	9.0	7.6	4.7	90.2	12.4	7.0	4.0
93.5	13.6	99.7	8.5	6.2	5.1	93.2	13.0	6.5	4.5
85.5	13.9	92.9	9.0	7.4	4.9	87.0	12.2	5.9	3.2
92.0	13.6	98.7	8.5	6.7	5.1	92.0	12.8	6.7	4.3
84.1	13.7	91.0	8.9	6.9	4.8	86.0	12.3	5.0	3.4
Average				7.01	4.81				
								6.65	3.86

The effect of change in moisture content from 4% to 18% was studied on three samples of flour. The results obtained on one of the three samples are recorded in Figure 1; the other two samples gave similar results. As the moisture content decreased from 18% to 8%, there was a very rapid increase in weight per given volume. The maximum weight was reached at a point between 8% and 7% moisture, and with further loss of moisture there was a decrease in weight per given volume.

A cupful of water weighs 236.6 gms., while a cupful of flour weighs approximately 92 gms. The gain in weight with loss in moisture, shown in Figure 1, would indicate a physical change in the size of the flour granules. The effect of change in moisture content of flour on apparent density of flour and weight of water was calculated from Figure 1 and recorded in Figure 2.

Effect of Relative Humidity

That the moisture of flour varies according to the temperature and humidity of the surrounding medium is the daily observation of all

engaged in cereal investigations. Flour high in moisture content was found to lose moisture and gain in weight per cup of flour when sifted in a room of low relative humidity, while flour of low moisture content

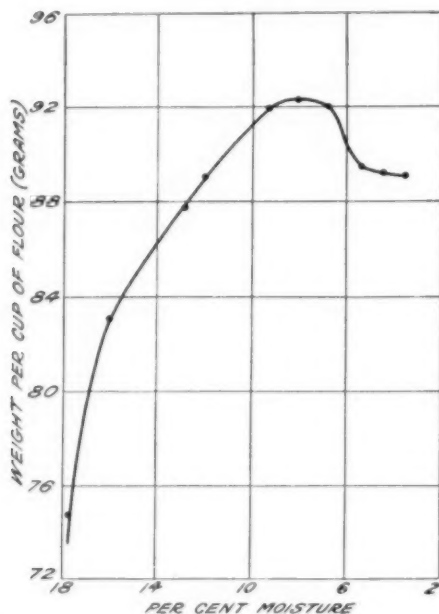


Fig. 1. Effect of change in moisture content on weight per cup of flour.

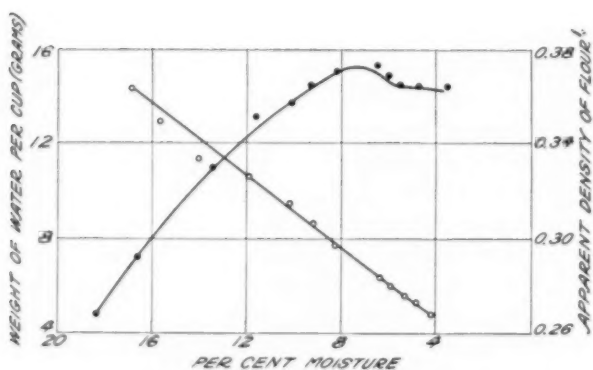


Fig. 2. Effect of change in moisture content of flour on apparent density of flour and weight of water per cup of flour.

$$^1 \frac{\text{Weight of flour in cup (gms.)} - \text{Weight of water in flour (gms.)}}{\text{Volume of cup (cc.)}}$$

gained in moisture and lost in weight per given volume when sifted in a room of fairly high relative humidity (Tables V and VI).

TABLE V

EFFECT OF LOW RELATIVE HUMIDITY, SUCH AS IS FOUND IN HEATED ROOMS IN WINTER, ON WEIGHT PER GIVEN CUP WHEN THE FLOUR IS HIGH IN MOISTURE

	Weight per given cup of flour	Moisture
	Gms.	P.ct.
Original		17.2
After first sifting	86.0	16.8
After second sifting	87.4	16.6
After third sifting	87.9	16.4
After fourth sifting	89.9	16.3

TABLE VI

EFFECT OF FAIRLY HIGH RELATIVE HUMIDITY, SUCH AS IS COMMON IN SUMMER, ON WEIGHT OF FLOUR PER GIVEN MEASURE WHEN THE FLOUR IS LOW IN MOISTURE. (RELATIVE HUMIDITY 65%)

	Flour sample							
	No. 1		No. 2		No. 3		No. 4	
	Moisture	Weight per given cup	Moisture	Weight per given cup	Moisture	Weight per given cup	Moisture	Weight per given cup
Original	P.ct.	Gms.	P.ct.	Gms.	P.ct.	Gms.	P.ct.	Gms.
After 1st sifting	9.60	88.3	9.68	107.5	9.70	106.4	9.67	92.3
After 2d sifting		87.2		106.6		106.1		90.5
After 3d sifting		86.9		107.2		104.4		90.0
After 4th sifting		85.8		107.2		104.4		89.5
After 5th sifting	10.17	85.5	10.16	106.0	10.20	104.1	10.15	89.3

Equipment was not available for experiments on relative humidity. From the data at hand, it seems probable that flour when sifted loses or gains in weight according to the relation of its moisture content to the relative humidity of the surrounding atmosphere in accordance with the findings of Bailey (1920), who observed that flours exposed to different atmospheric conditions ranged from 5% moisture at 30% relative humidity to 15.5% moisture at 80% relative humidity at 25° C.

Flour of low moisture content was placed on a cover glass with the flour surface above water contained in a drop slide. The flour was examined by a microscope for change in size by immediately locating one flour granule on the micrometer scale and taking measurements. Measurements were taken a second time after the flour had had time to absorb moisture. It was found that dry flour granules increased in size when placed in a moist atmosphere.

Effect of Temperature on the Weight of a Cup of Flour

Ten samples of flour were transferred to pint Mason jars; the jars were sealed and placed in a constant temperature cabinet maintained at 20° C. Two days later the flour was tested for weight per given volume in a room maintained at 20° C. Moisture determinations were made on the flour following the test for weight per given volume. The data obtained by these two procedures are recorded in the first and second columns of Table VII. The flour was returned to the container, sealed and placed in a constant temperature cabinet maintained at 30° C. After two days the flour was again tested for weight per given volume and moisture. This time the work was done in a room maintained at 30° C. The data obtained at this time appear in the third and fourth columns of this table. The flour weighed heavier per given volume at the lower temperature; there was an average change in weight of 2.46 gms. per cup of flour. This work was done in winter in a room of low relative humidity. Sifting at the higher temperature caused the flour to lose a small quantity of moisture. It has been shown in Table IV that as flour loses in moisture it gains in weight per given volume. Were it not for this loss in moisture, the difference in weight per given volume would be greater at the two temperatures.

TABLE VII
EFFECT OF DIFFERENT TEMPERATURES ON THE WEIGHT OF A CUP OF FLOUR
(20° C.-30° C.)

20° C.		30° C.		Change in weight per cup	Change in moisture
Weight per cup	Moisture	Weight per cup	Moisture		
Gms.	P.ct.	Gms.	P.ct.	Gms.	P.ct.
95.7	10.00	94.1	9.82	1.6	0.18
94.0	10.33	91.5	10.23	2.5	0.10
96.3	10.19	92.9	9.93	3.4	0.26
102.5	10.12	100.0	9.89	2.5	0.23
107.1	10.30	103.5	10.00	3.6	0.30
99.6	10.10	97.9	9.81	1.9	0.29
97.0	10.10	95.4	9.82	1.6	0.28
98.9	10.60	96.1	10.18	2.8	0.41
92.8	10.20	90.6	9.80	2.2	0.40
95.4	10.35	92.9	9.85	2.5	0.40

Discussion

Effect of size of flour granules had been the subject of innumerable investigations and its importance has been established for both bread and pastry purposes. Weight per given volume when determined under standard conditions will give a fairly accurate indication of the mill run at any one time as compared to that of another time and will assist the miller in judging the uniformity of his flour.

Effect of variation in moisture content is a very important factor. Flour as it comes from the mill has a moisture content of about 13.5%. In summer, when the humidity is high, there may be a gain in moisture. Flour retained in heated homes in winter tends to establish an equilibrium with the low relative humidity of the atmosphere and lose moisture. With loss of moisture there is a physical change in the flour which results in more flour per given volume. This loss in moisture and change in weight both adversely affect the ratio of flour to liquid. The resulting error in the amount of flour taken when flour is measured by volume is sufficient to account for some of the failures in cake making. Milling companies which enclose recipes with their flour using volume measurement could assist their customers by making modifications in their recipes so as to take care of these changes in relative humidity of homes.

Blends of soft and hard wheat flour are frequently sold as pastry flours. Recipes adapted to either hard or soft wheat flour will prove unsatisfactory with blends unless the proportions are changed, as there is not only a difference in weight, but also in the factor of absorption. There are seasons in which the soft wheat flour supply is small and the miller must necessarily manufacture blends of soft and hard wheat flours. The miller who changes from a soft wheat flour to a blend without calling attention to the proper proportions to use, causes difficulties in the use of his flour, for pastry flour made from a blended flour requires more liquid than that made from a soft wheat flour.

Throughout the entire work all commercially milled flours made from soft wheat which were examined weighed less than 100 gms. per cup and all hard wheat flours weighed more than 105 gms. per cup. Weight per given volume may be used as a simple and fairly accurate means of distinguishing between soft wheat flour, hard wheat flour, and blends.

Summary

Effect of size of flour granule was measured in two ways: (1) By securing separations with different sized sieves, and (2) by using flour separated into grades. Granulation was found to be a very important factor in weight per cup.

Variation in moisture content is an important factor. As flour loses moisture it gains in weight per given volume and vice versa. This would indicate a physical change in the size of the flour particle.

Relative humidity of the room in which volume determinations were made is a factor because of its effect on the moisture content of the flour.

Temperature of the room in which volume determinations were made affects the weight of flour per given volume. The flour weighed more per cupful at 20° C. than at 30° C.

Blends of equal weights of two flours weighed approximately the same as the average weight of the two flours used in making the blend.

Acknowledgment

The author wishes to express her appreciation to the following for assistance in supplying samples of flour: Cammander-Larabee Corporation, Mennel Milling Company, Booneville Mills Company.

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A NOTE ON AN EFFECT OF METAL IN MIXING BREAD DOUGHS

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(Received for publication February 23, 1932)

In the course of a series of baking experiments, it was considered desirable to compare the results with several flours using machine mixed doughs as well as the usual hand mixed ones. As the laboratory is equipped with a bronze mixer which has a normal capacity of 300 gms. of flour, and which is motor driven at constant speed, it was thought to be suitable and its use was attempted for the purpose.

The baking procedure employed was the tentative method of the A. A. C. C. with two modifications—addition of 2% of lard, and light greasing of the baking pans. Lard was added to the dough after the 62nd cut, if hand mixed; or at the end of 2 minutes, if machine mixed. After machine mixing, the dough was divided at once into three equal portions for fermentation and baking.

A series of preliminary tests seemed to indicate that five minutes mixing with the machine was the minimum period for the production of a smooth dough and of maximum loaf volume. These maximum fig-

ures, however, contrary to the findings of Merritt and Blish (*Cereal Chem.* 8: 267-292) were distinctly smaller than loaf volumes obtained with corresponding hand mixed doughs.

A series of daily baking tests, extending over a period of several weeks, with doughs mixed for five minutes in the bronze mixer, gave data very low in correlation values. Toward the end of this series, the mixer, through an oversight, was allowed to stand overnight with a dough in it consisting of flour and water only, in place of being cleaned at once, as ordinarily, and allowed to stand approximately 20 hours before being used again. In this instance it was scraped free of the dough mass, washed with tap water and dried with a towel for immediate use. The surface of the metal was visibly discolored. The three loaves from the first dough mixed averaged 40 cc. smaller in volume than six loaves, from the same flour, mixed as first doughs, respectively, on the two preceding days. As the average volume of these six loaves was itself approximately 40 cc. below a normal figure, attention was drawn to the possibility of contamination.

A review of the data previously referred to indicated that the volume figures for loaves from the first dough mixed each day were distinctly smaller than for those from succeeding doughs from the mixer on the same day.

To demonstrate this apparent contact effect, a series of loaves was baked from a spring wheat flour with analysis of .45% ash, and 14.60% protein. Twelve loaves, three from the first dough from the mixer on each of four days respectively averaged 543 cc.; while the same number of loaves from the second doughs mixed, after lapse of but sufficient time for the mixer to be lightly scraped, washed, and dried, averaged 578 cc. in volume. Twelve loaves from hand mixed doughs, as controls, averaged 615 cc.

These are taken to be indications that the surface of this metal takes on a coating upon standing in contact with air which is not removed by washing with water, but is largely removed by the first dough mixed thereafter. No work has been done in this laboratory to determine the possible effects of iron, tin, copper, aluminum, etc., under the same conditions.

BOOK REVIEWS.

The Methods of Statistics. By L. H. C. Tippett, M.Sc. (Lond.), Statistician to the British Cotton Industry Research Association. Published by Williams and Norgate Ltd., 38 Great Ormond Street, London, W.C. 1, 1931. Demy 8vo, 222 pp., 12 diagrams. Price 15 s. net.

Designated as "An introduction mainly for workers in the biological sciences," this volume has more to commend it than any other single work in English addressed to the needs of the non-mathematically trained mind which must deal with the task of precise interpretation of variable phenomena. While the author aims essentially to present statistical methods of tested utility in such a fashion that they may be applied with confidence by the biologist to his own particular problems, an attempt is made to include sufficient explanation in non-technical terms of the underlying reasoning that he may attack his statistical work with an intelligent appreciation of the usefulness of the methods employed. In the latter attempt, Tippett will undoubtedly prove to have been more successful than his predecessors in statistical text-book writing. There is, however, still room for improvement in this most important part of the presentation.

In the preface the author points out that he has attempted to present "a single system of statistics" combining the newer theories of Dr. R. A. Fisher in an harmonious blending with the older classical contributions of Prof. Karl Pearson and his associates. Although his achievement in that direction is commendable, his enthusiasm for the "analysis of variance" has led him to fail to give adequate recognition of alternative procedures of established validity for large samples. The limitations of small samples as bases of generalization do not appear to have been adequately treated.

The author indulges in apt simile when he writes: "Statistics is a tool, and the good craftsman knows what he wants to make with his tool before he lifts it; it is only the small boy who uses it promiscuously. In this book I have often been like the small boy, but if my readers will be small boys with me, they will learn how to handle the tool, and then will be able to apply it with biological judgment." The preface closes with the distressing but wise injunction "There is no better way of learning statistics than by working through examples," to which the reviewer must subscribe his "Amen."

Presenting briefly a few definitions and ideas in an Introduction, the author quickly passes to a terse discussion of frequency distributions and their statistical description. His expulsion of irrelevant technicalities (i.e. to biologists) in this chapter is commendable. However, since it forms so important a foundational section of the whole statistical structure, the reviewer believes Tippett would have considerably enhanced the value of his book by elaboration in discussion and biological illustration of the several topics dealt with. Nevertheless, the reader who carefully weighs every sentence in this chapter for its import will find himself equipped with a satisfactory digest of essential principles from which he may go on to an intelligent study of the remainder of the book.

It is not necessarily advantageous to the prospective reader that detailed comment upon the individual chapters that follow should be given here. Suffice it to say that, in all, the field of practical statistical analysis is reasonably well covered without restricting the methods to those of any one school of thought. The reviewer regrets that the broad view of correlation analysis becomes somewhat lost in a discussion of the component "variances" of correlation surfaces.

Much of the beauty of mathematical procedures lies in the economy of human thought made possible through generalization in the form of symbolic reasoning. Unfortunately that form of reasoning is at the same time the despair of biologists who for years have been dissociated from it. Recognizing this, Tippett refrains from much indulgence in it, but it is well to remember that no worthwhile text in statistics could be written without it. The fault lies not in the method. The cereal chemist who will be patient and diligent in breaking himself into the task of grasping a subject which appears to be difficult solely because it is new to him will find much to reward

his labors in Tippet's book. Citation to original sources might profitably have been extended and some chapters expanded, but details that cloud instead of broaden the vision are commendably absent.

ALAN E. TRELOAR.

Mikrophotographischer Atlas der Zerealien Einschliesslich Hirsen und Buchweizen Sowie der Wichtigsten Leguminosen. By D. Strilciuc. Published by Der Zeitschrift für das Gesamte Getreide- und Mühlenwesen, Berlin N. 65, Seestr. 11, 1932. 16 x 24 cm. paper, 85 pp., 284 illustrations. Price 6.50 R.M.

This booklet consists of many series of excellent microphotographs illustrating the important cell structures to be found in wheat, rye, barley, buckwheat, oats, corn, rice, the grain sorghums, Italian millet, and other cereals. A similar extensive series of microphotographs are also given for 16 of the more important legumes. Over 80 per cent of the booklet is devoted to the 284 microphotographs presented. Great care has been taken in the preparation and selection of the material for photographic purposes. The photographing and enlarging of the microphotographs have been especially well done to the extent that differences in cell structure are plainly visible.

A worth while introduction describes the procedure for the preparation of material when using the Atlas as a guide.

The booklet should be a valuable addition to the library of the microscopist, the botanist, and those cereal chemists, especially feed control chemists, who concern themselves with the microscopy of cereals and feeding stuffs.

D. A. COLEMAN.

ERRATA

Report of Activities of A. A. C. C. Baking Research Fellowship, P. P. Merritt, M. J. Blish, and R. M. Sandstedt. Page 192, line 4 from bottom, for "5/8" read "5/16."

Sugars, Diastatic Activity, and "Gassing Power" in Flour, M. J. Blish, R. M. Sandstedt, and G. R. Astleford. Page 392, line 20, for "elimination" read "estimation."

INDEX—AUTHOR AND SUBJECT

	Page
Acidity	
Development of, in bread doughs. P. Halton and E. A. Fisher.	34
Effect of, on accuracy of maltose determinations. M. J. Blish, R. M. Sandstedt, and G. R. Astleford.	383
Grades of flour. P. Halton and E. A. Fisher.	6
in flours, Methods for determining. A. Schulerud.	128
of bread. Yoshitaka Hashitani and Tsunenaka Sako.	115
of bread doughs. P. Halton and E. A. Fisher.	1, 3, 4
of bread flours due to bleaching. W. H. Hanson.	376
of cake flours. E. E. Smith.	425
of crackers in relation to keeping quality. H. O. Triebold and C. H. Bailey	102
Volatile and non-volatile, in bread doughs. M. J. Blish and R. C. Hughes	346
Agene, Flour bleaching studies with. W. H. Hanson.	360
Aging of flour, Relation of bleaching agents to speed of. W. H. Hanson.	358
Alcohol in bread doughs. M. J. Blish and R. C. Hughes.	331
Alexander, Geo. L.	
A note on the value of the viscosity test for determining some of the properties of cake and pastry flours.	169
Chairman, Report of subcommittee on viscosity of cake flours.	428
Ammonium phosphate, Effect of, on development of pH and CO ₂ during bread dough fermentation. P. Halton and E. A. Fisher.	38
Amylases and hydrolysis of starch in bread. A. S. Schultz and Quick Landis.	305
Analysis of variance in cereal chemistry experiments. C. H. Goulden.	239
Apparatus	
for determining gassing powers of flours. M. J. Blish, R. M. Sandstedt, and G. R. Astleford.	389
for making oxidation studies on fat. H. O. Triebold and C. H. Bailey.	52
for measuring gas production and expansion in doughs. M. C. Markley and C. H. Bailey.	591
for studying gas production in doughs. W. L. Heald.	603
Ash in flour	
Influence on viscosity measurements of. C. E. Rich.	535
Report of subcommittee on, magnesium-acetate-alcohol quick ash procedure. C. F. Davis.	431
Ashing of flours. J. W. Bowen.	158
Association of Official Agricultural Chemists, Report of general referee on cereal foods, 1931. J. A. LeClerc.	83
Astleford, G. R., M. J. Blish, and R. M. Sandstedt. Sugars, diastatic activity and gassing power in flour.	378
Atmospheric conditions and heating of feed grains. H. J. Smith and J. Paul Bartz.	393
Automatic method for measuring gas production and expansion in doughs. M. C. Markley and C. H. Bailey.	591
Bailey, C. H.	
Book review. Colloid aspects of food chemistry and technology, by William Clayton.	172
and M. C. Markley. An automatic method for measuring gas production and expansion in doughs.	591
and Oscar Skovholt. Effect of temperature and of the inclusion of dry skim milk upon properties of dough as measured with the farinograph.	523
A. E. Treloar, and R. C. Sherwood. Some relationships involving crumb, texture, and color.	121
and H. O. Triebold. A chemical study of rancidity, I, II.	50, 91
Bailey, L. H.	
Report of subcommittee chairman on cake baking method for soft wheat flours.	407
The use of certain constituents in breadmaking with particular reference to the problem of staling.	65

	Page
Baking quality, Physical and chemical properties of ether-soluble constituents of wheat flour in relation to. William McK. Martin and W. O. Whitcomb..	275
Baking strength of garnet wheat. C. E. Rich.....	540
Relation of diastatic activity to. L. Elion.....	86
Baking test as a measure of the quality of bread wheats. C. O. Swanson and E. H. Kroeker.....	13
Collaborative studies on wheat varieties. C. O. Swanson and E. H. Kroeker.....	26
Report of committee on standard test. D. A. Coleman, Chairman.....	404
Barackman, R. A., Chairman. Report of subcommittee on methods of scoring cake.....	409
Bartz, J. Paul, and H. J. Smith. Heating of feed grains.....	393
Bayfield, E. G. Soft winter wheat improvement program for Ohio.....	322
Benzoyl peroxide, Flour bleaching studies with. W. H. Hanson.....	358
Beta Chloral, Flour bleaching studies with. W. H. Hanson.....	358
Biscuit flours, Report of subcommittee on tests for. L. D. Whiting, Chairman	414
Bleaching, Resistance of flours to. C. O. Swanson and E. H. Kroeker.....	26
Blish, M. J. Chairman, Report of nominating committee.....	443
and R. C. Hughes. Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance.....	331
R. M. Sandstedt, and G. R. Astleford. Sugars, diastatic activity and gassing power in flour.....	378
R. M. Sandstedt, and P. P. Merritt. Report of the activities of the A. A. C. C. baking research fellowship.....	175
Book reviews Alcoholic fermentation, fourth edition, by A. Harden; reviewed by E. Ziegler.....	174
Colloid aspects of food chemistry and technology, by William Clayton; reviewed by C. H. Bailey.....	172
Das Getreidekorn, by J. F. Hoffmann and K. Mohs; reviewed by J. A. LeClerc.....	173
Introduction to agricultural biochemistry, by R. Adams Dutcher and Dennis E. Haley; reviewed by R. A. Gortner.....	171
Mehlchemie, third edition, by K. Mohs; reviewed by E. Ziegler.....	174
Mikrophotographischer Atlas der Zerealien, by D. Strilciuc; reviewed by D. A. Coleman.....	639
Official and tentative methods of analysis of the Association of official agricultural chemists, third edition; reviewed by D. A. Coleman.....	89
The law of patents for chemists, by Joseph Rossman; reviewed by E. S. Stateler.....	328
The methods of statistics, by L. H. C. Tippett; reviewed by A. E. Treloar	638
The structure and composition of foods, I, cereals, nuts, oil seeds, by Andrew L. and Kate Barber Winton; reviewed by S. J. Lawellin.....	329
Bowen, J. W. A method for quick ashing of flour.....	158
Brabender, C. W. Studies with the farinograph for predicting the most suitable types of American export wheats and flours for mixing with European soft wheats and flours.....	617
Bread Effect of flour bleaching on characteristics of. W. H. Hanson.....	357
Hydrogen-ion concentration of. Yoshitaka Hashitani and Tsunenaka Sako	115
Staling of. L. H. Bailey.....	65
Vitamin B content of. Yoshitaka Hashitani and Tsunenaka Sako.....	107
Brooke, Mary M., Chairman. General report of committee on methods of testing cake and biscuit flours.....	406
Brown, Pearl, Chairman. Report of subcommittee on tests of soft wheat flour for use in cookies.....	420
Bulletin reviews Bibliography of bibliographies on chemistry and chemical technology, second supplement, compiled by C. J. West and D. D. Berolzheimer; reviewed by D. A. Coleman.....	377

	Page
Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins, U. S. D. A. Circ. 183C, by D. B. Jones; reviewed by D. A. Coleman	33
Fifth annual report (1931), Dominion Grain Research Laboratory (Canada), F. J. Birchard; reviewed by D. A. Coleman	448
Report of operation, state testing mill, crop season 1930, by H. A. Halvorson; reviewed by D. A. Coleman	330
Second annual report, Wheat Research Institute (New Zealand) Bulletin No. 35; reviewed by D. A. Coleman	448
The use of oils, fats, and emulsions of oils and fats in bread making, by E. A. Fischer and C. R. Jones (Natl. Assocn. Rev., Apr. 1932); reviewed by D. A. Coleman	448
Bunzell, H. H., and Marjorie Kenyon. On rope control	161
Cake flour	
General report of committee on methods of testing. Mary M. Brooke, Chairman	406
Relation of flour bleaching to quality of. E. E. Smith	425
Report of subcommittee on cake baking method for soft wheat flour. L. H. Bailey, Chairman	407
Report of subcommittee on methods of scoring. R. A. Barackman, Chairman	409
Viscosity test for evaluating some of the properties of. G. L. Alexander	169
Carbon dioxide	
Development of, in bread doughs. M. J. Blish and R. C. Hughes	344
Development of, in bread doughs. P. Halton and E. A. Fisher	34
Catalyst	
Chemical study of rancidity, I, II. H. O. Triebold and C. H. Bailey	50, 91
Metallic selenium, in Kjeldahl method. H. C. Messman	357
Selenium oxychloride, in Kjeldahl method. C. E. Rich	118
Chlorine	
and quality of cake flours. E. E. Smith	424
(Beta Chloro) in relation to flour bleaching. W. H. Hanson	358
Coe, Mayne R., and J. A. LeClerc. Photochemical action a cause of rancidity	519
Coleman, D. A.	
Book reviews	
Mikrophotographischer Atlas der Zerealien, by D. Strilciuc	639
Official and tentative methods of analysis of the Association of Official Agricultural Chemists, third edition	89
Bulletin reviews	
Bibliography of bibliographies on chemistry and chemical technology, second supplement, compiled by C. J. West and D. C. Berolzheimer	377
Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins, U. S. D. A. Circ. 183C, by D. B. Jones	33
Fifth annual report (1931), Dominion Grain Research Laboratory (Canada), F. J. Birchard	448
Second annual report, Wheat Research Institute (New Zealand) Bulletin No. 35	448
The use of oils, fats, and emulsions of oils and fats in bread making, by E. A. Fisher and C. R. Jones (Natl. Assocn. Rev., Apr. 1932)	448
Chairman, Report of committee on the standard baking test	404
Report of Editor-in-chief of Cereal Chemistry	439
Color of crumb	
and loaf volume. A. E. Treloar, R. C. Sherwood, and C. H. Bailey	123
and protein in flour. A. E. Treloar, R. C. Sherwood, and C. H. Bailey	123
and texture of crumb. A. E. Treloar, R. C. Sherwood, and C. H. Bailey	124
Committees of the A. A. C. C. for 1932	444, 445
Composition of brown and polished rice. J. A. LeClerc	601
Composition of wheat	
Effect of climate on. H. R. Kraybill	74
Effect of nutrition on. H. R. Kraybill	73, 76
Cookie flours, Report of the subcommittee on tests for. Pearl Brown, Chairman	420

	Page
Cooking qualities of domestic rices. Mabel C. Stienbarger.....	317
Corn, Conditions responsible for spoilage in storage of. H. J. Smith and J. Paul Bartz.....	393
Crackers, Keeping quality of. H. O. Triebold and C. H. Bailey.....	57
Davis, C. F., Report of collaborative studies on the magnesium-acetate-alcohol method.....	431
Determination of acidity in flours. A. Schulerud.....	128
Diastatic activity of flour. M. J. Blish, R. M. Sandstedt, and G. R. Astleford.....	386
Diastatic activity and gassing power in flour. M. J. Blish, R. M. Sandstedt, and G. R. Astleford.....	391
Dibenzoyl peroxide, Bleaching properties of, as influenced by mercury vapor lamp. C. G. Ferrari, Alice B. Croze, and C. H. Bailey.....	491
Dough	
Automatic method for measuring expansion of. M. C. Markley and C. H. Bailey.....	591
Determination of alcohol in. M. J. Blish and R. C. Hughes.....	331
Determination of hydrogen-ion concentration of. M. J. Blish and R. C. Hughes.....	331
Determination of total acidity in. M. J. Blish and R. C. Hughes.....	331
Determination of volatile and non-volatile acids in. M. J. Blish and R. C. Hughes.....	331
Development of hydrogen-ion concentration in, during fermentation. P. Halton and E. A. Fisher.....	1
Fermentation tolerance studies with. C. O. Swanson and E. H. Kroeker.....	140
Hydrogen-ion concentration values of, made from different grades of flour. P. Halton and E. A. Fisher.....	1
Dough breaks, effect on fermentation tolerance. C. O. Swanson and E. H. Kroeker.....	139
Dough fermentation by-products. M. J. Blish and R. C. Hughes.....	331
Dough, punching of	
Effect of, on H-ion concentrations. P. Halton and E. A. Fisher.....	39
Effect of, on gas production. P. Halton and E. A. Fisher.....	39
Dunlap, F. L.	
Chairman, Rosenwald Museum of Science Committee.....	441
Observations on nutrition.....	289
Durham, R. K., President's address.....	402
Effect of metal in mixing bread doughs. E. N. Frank.....	636
Effect of plant nutrition on the composition of wheat. H. R. Kraybill.....	71
Effect of temperature on plasticity of bread dough. Oscar Skovholt and C. H. Bailey.....	523
Effect of temperature and of the inclusion of dry skim milk upon the properties of doughs as measured by the farinograph. Oscar Skovholt and C. H. Bailey.....	523
Effects of varying sugar concentrations in bread doughs on fermentation by-products and fermentation tolerance. M. J. Blish and R. C. Hughes.....	331
Elion, L. A note on the separation of diastatic activity from strength in baking tests.....	83
Errata	
Report of activities of A. A. C. C. baking research fellowship. P. P. Merritt, M. J. Blish, and R. M. Sandstedt.....	640
Sugars, diastatic activity, and gassing power in flour. M. J. Blish, R. M. Sandstedt, and G. R. Astleford.....	640
Testing wheat varieties for milling and baking quality. C. O. Swanson and E. H. Kroeker.....	330
Ether	
Effect of extraction with, on bread making properties of wheat flour. Wm. McK. Martin and W. O. Whitcomb.....	275
soluble constituents of flour, Physical and chemical properties of. Wm. McK. Martin and W. O. Whitcomb.....	279
Experimental milling tests, Limitations of. C. O. Swanson and E. H. Kroeker.....	11
Factor for converting nitrogen to crude protein in wheat and bran. G. L. and W. Kedzie Teller.....	571

	Page
Factors	
influencing the rate of absorption of water by wheat. C. W. Fraser and W. H. Haley	45
which affect gas production during dough fermentation. W. L. Heald	603
Farinograph	
Use of, in connection with flour blending. C. W. Brabender	617
Use of, in connection with skimmilk studies with bread doughs. Oscar Skovholt and C. H. Bailey	523
Feed grains, Proper storage for. H. J. Smith and J. Paul Bartz	393
Fermentation by-products, Effect of sugar on. M. J. Blish and R. C. Hughes	331
Fermentation tolerance	
as affected by addition of lactic acid to dough. C. O. Swanson and E. H. Kroeker	144
as affected by use of dough breaks. C. O. Swanson and E. H. Kroeker	141
as affected by sugar supply of dough. M. J. Blish and R. C. Hughes	331
as affected by sugar supply of dough. C. O. Swanson and E. H. Kroeker	142
as affected by remixing of dough. C. O. Swanson and E. H. Kroeker	139
as affected by repeated punching of doughs. C. O. Swanson and E. H. Kroeker	141
Fifield, C. C. Report of Managing Editor of Cereal Chemistry	440
Fischer, V. E. Chairman, Report of auditing committee	443
Fisher, E. A. and P. Halton	
Observations on the determination of H-ion concentration of dough and on the relation of H-ion concentration to flour grade	1
Significance of H-ion concentration in panary fermentation	34
Flour	
biscuit, Tests for quality of. L. D. Whiting	414
bread, Tests for quality of. M. J. Blish, R. M. Sandstedt, and P. P. Merritt	175
cake, Tests for quality of. L. H. Bailey	407
Diastatic activity of. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	378
Diastatic activity of. W. L. Heald	603
Diastatic activity of. C. O. Swanson and E. H. Kroeker	137
Gassing power of. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	378
Hydrogen-ion concentration of various grades of. P. Halton and E. A. Fisher	6
pie, Tests for quality of. C. B. Kress	411
Reducing sugars in. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	378
Sucrose content in. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	378
Flour acidity	
Determination of hydrogen-ion concentration. P. Halton and E. A. Fisher	1
Influence of bleaching agents on. W. H. Hanson	377
Titration methods for determining. A. Schulerud	128
Flour bleaching	
Chemical changes incident to. W. H. Hanson	376
with benzoyl peroxide. W. H. Hanson	358
with chlorine (Beta Chloral). W. H. Hanson	358
with chlorine and development of hydrogen-ion concentration. E. E. Smith	424
with nitrogen-trichloride. W. H. Hanson	358
Frank, E. N., Effect of metal in mixing bread doughs	636
Fraser, C. W. and W. L. Haley. Factors that influence the rate of absorption of water by wheat	45
Freeman, M. E. and R. A. Gortner. The gums of the cereal grains	506
Garnet wheat, Baking strength of. C. E. Rich	540
Gas production in dough	
Effect of ammonium phosphate on. P. Halton and E. A. Fisher	38
Effect of punching (knocking back) on development of. P. Halton and E. A. Fisher	39
Effect of malt extract on. P. Halton and E. A. Fisher	38
Effect of mixing time on. W. L. Heald	613
Effect of salt concentration on. W. L. Heald	608

	Page
Gas production in dough	
Effect of sucrose on. P. Halton and E. A. Fisher	38
Effect of sugar on. W. L. Heald	609
Effect of temperature on. W. L. Heald	614
Effect of water absorption on. W. L. Heald	610
Effect of yeast foods on. W. L. Heald	611
Method for automatic measurement of. M. C. Markley and C. H. Bailey	591
Relation of pH to. P. Halton and E. A. Fisher	35
Gassing power of flour, Method for determining. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	388
Gortner, R. A.	
Book review. Introduction to agricultural biochemistry, by R. Adams Dutcher and Dennis E. Haley	171
and M. E. Freeman. The gums of the cereal grains	506
Goulden, C. H. Application of the variance analysis to experiments in cereal chemistry	239
Grewe, Emily. Variation in the weight of a given volume of different flours, I, II, III	311, 531, 628
Gums of the cereal grains. Monroe E. Freeman and Ross Aiken Gortner	506
Gustafson, C. B. Acid and iodine numbers of the oil from soft winter wheat	595
Haley, W. L. and C. W. Fraser. Factors that influence the rate of absorption of water by wheat	45
Halton, P. and E. A. Fisher	
Observations on the determination of H-ion concentration of dough and on the relation of H-ion concentration to flour grade	1
Significance of H-ion concentration in panary fermentation	34
Hanson, W. H. Effects of the amount and kind of bleach used on flour in relation to its bleaching	358
Harris, R. H. Relation between total protein, peptizable protein, and loaf volume as obtained by successive increments of $KBrO_3$	147
Hashitani, Yoshitaka and Tsunenaka Sako. On the vitamin B content of bread	107
Heald, W. L. Some factors which affect gas production during dough fermentation	603
Heating of feed grains. H. J. Smith and J. Paul Bartz	393
Hughes, R. C. and M. J. Blish. Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance	331
Hydrogen-ion concentration	
Development of, during dough fermentation. P. Halton and E. A. Fisher	34
Development of, in bleached flours. W. H. Hanson	376
Development of, in bread doughs. M. J. Blish and R. C. Hughes	338
Effect of flour concentration on pH of flour-water mixtures. P. Halton and E. A. Fisher	6
Effect of KH_2PO_4 on pH values. P. Halton and E. A. Fisher	7, 8
Effect of punching (knocking back) on. P. Halton and E. A. Fisher	39
of bread. Yoshitaka Hashitani and Tsunenaka Sako	115
of cake flours. E. E. Smith	425
Relation of, to grade of flour	8
Relation of, to keeping quality of crackers	102
Hydrolysis of starch in bread by flour and malt amylase. A. S. Schultz and Quick Landis	305
Induction period and keeping quality of fat and crackers. H. O. Triebold and C. H. Bailey	58
International bread-making congress	90
Iodine number of wheat oil. C. B. Gustafson	597
Keeping qualities of crackers	
Effect of H-ion concentration on. H. O. Triebold and C. H. Bailey	102
Effect of re-heating on. H. O. Triebold and C. H. Bailey	101
Effect of storage in CO_2 on. H. O. Triebold and C. H. Bailey	100
Effect of temperature on. H. O. Triebold and C. H. Bailey	102
Keeping qualities of shortenings	
Chemical characteristics of, as indices of. H. O. Triebold and C. H. Bailey	94

	Page
Effect of storage conditions on. H. O. Triebold and C. H. Bailey.....	91
Method for the determination of. H. O. Triebold and C. H. Bailey.....	51
Kenyon, Marjorie and H. H. Bunzell. On rope control.....	161
Kraybill, H. R. Effect of plant nutrition on the composition of wheat.....	71
Kress, C. B., Chairman. Report of subcommittee on pie flour tests.....	411
Kroecker, E. H. and C. O. Swanson	
Some factors in fermentation tolerance.....	137
Testing wheat varieties for milling and baking qualities.....	10
Landis, Quick and A. S. Schultz. Hydrolysis of starch in bread by flour and malt amylase.....	305
Lawellin, S. J., Book review. The structure and composition of foods, I, cereals, nuts, and oil seeds, by Andrew L. and Kate Barber Winton.....	329
Leatherock, L. E., Chairman, Report of employment committee.....	442
LeClerc, J. A.	
Book review. Das Getreidekorn, by J. F. Hoffmann and K. Mohs.....	173
Comparative composition of brown and polished rice—losses in material due to polishing.....	601
Referee, Report of cereal section of the Association of Official Agricultural Chemists, 1931.....	83
and Mayne R. Coe. Photochemical action, a cause of rancidity.....	519
Light in relation to development of rancidity in cereal foods. Mayne R. Coe and J. A. LeClerc.....	519
Loaf volume	
Action of successive increments of KBrO ₃ on. R. H. Harris.....	150
and crumb color. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	122, 123
and fermentation time. M. J. Blish and R. C. Hughes.....	347
and flour protein peptization. R. H. Harris.....	150
and percentage of sugar. M. J. Blish and R. C. Hughes.....	347
and protein content of flour. R. H. Harris.....	150
and protein content of flour. C. E. Rich.....	540
and protein content of flour. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	123, 124
and texture scores. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	123, 124
and viscosity of flour in water suspensions. C. E. Rich.....	540
Effect of bleaching on. W. H. Hanson.....	358
Logue, Paul, Chairman. Report of the committee on resolutions.....	442
Magnesium-acetate-alcohol method for ashing flours. C. F. Davis.....	431
Malt amylase and hydrolysis of starch in bread. A. E. Schultz and Quick Landis.....	305
Malt extract, Effect of, on development of pH and CO ₂ during bread dough fermentation. P. Halton and E. A. Fisher.....	38
Mangels, C. E.	
Chairman, Report of committee on methods of analysis.....	429
Regional and seasonal variation in pigmentation of durum wheats.....	485
Markley, M. C. and C. H. Bailey. An automatic method for measuring gas production and expansion in doughs.....	591
Martin, Wm. McK. and W. O. Whitcomb. Physical and chemical properties of ether-soluble constituents of wheat flour in relation to baking quality.....	275
Material and mineral losses in milling rice. J. A. LeClerc.....	601
Materials retarding staling in bread. L. H. Bailey.....	67
Merritt, P. P., M. J. Blish and R. M. Sandstedt. Report of the activities of the A. A. C. C. baking research fellowship.....	175
Messman, Henry C. Metallic selenium as a catalyst in Kjeldahl digestions.....	357
Metallic selenium as a catalyst in Kjeldahl digestions. H. C. Messman.....	357
Methods	
Apparatus and procedure for studying heating of feed grains. H. J. Smith and J. Paul Bartz.....	393
Apparatus and procedure for studying oxidation of fats. H. O. Triebold and C. H. Bailey.....	50
for measuring gas production in dough. W. L. Heald.....	592
for measuring gas production and expansion in dough. M. C. Markley and C. H. Bailey.....	592
Magnesium-acetate-alcohol method for ash. C. F. Davis.....	431

	Page
Methods	
Quick ashing of flours. J. W. Bowen.....	158
Viscosity of flour in water suspensions. C. E. Rich.....	537
Viscosity test for evaluating cake flours. Geo. L. Alexander.....	169
Methods for the determination of	
acidity in flour. A. Schulerud.....	134
alcohol in bread doughs. M. J. Blish and R. C. Hughes.....	331
cake making properties of soft wheat flours. L. H. Bailey, Subcommittee Chairman.....	407
forms of nitrogen in wheat bran. G. L. Teller and W. Kedzie Teller.....	560
gassing power of flour. M. J. Blish, R. M. Sandstedt and G. R. Astleford.....	388
gums in cereal grains. M. E. Freeman and R. A. Gortner.....	506
hydrogen-ion concentration in doughs. M. J. Blish and R. C. Hughes.....	338
hydrogen-ion concentration in doughs and flour-in-water extracts. P. Halton and E. A. Fisher.....	1
maltose in flour. M. J. Blish, R. M. Sandstedt and G. R. Astleford.....	383
rope in bread. H. H. Bunzell and Marjorie Kenyon.....	163
sugar content of flour. M. J. Blish, R. M. Sandstedt and G. R. Astleford.....	378
total acidity in bread doughs. M. J. Blish and R. C. Hughes.....	341
viscosity of flour-in-water suspensions. E. E. Smith.....	426
vitamin B potency of bread. Yoshitaka Hashitani and Tsunenaka Sako.....	109
volatile and non-volatile acids in dough. M. J. Blish and R. C. Hughes.....	346
Methods, protein determinations	
Metallic selenium as a catalyst. Henry C. Messman.....	357
Selenium as a catalyst in. R. M. Sandstedt.....	156
Selenium oxychloride as a catalyst. C. E. Rich.....	118
Mitchell, R. W., Chairman, Report of history committee.....	441
Mixed feeds, Conditions responsible for spoilage in storage of. H. J. Smith and J. Paul Bartz.....	393
Mize, M. D., Secretary-Treasurer	
Minutes of eighteenth annual convention.....	438
Report of, for 1931.....	325
Moisture and heating of feed grains. H. J. Smith and J. Paul Bartz.....	393
Morison, C. B., Chairman, Report of employment committee.....	441
Nominating committee A. A. C. C., Report of. M. J. Blish, Chairman.....	443
Non-protein nitrogen compounds in cereals and their relation to the nitrogen factor for protein in cereals and bread. G. L. Teller.....	261
Oats, Conditions responsible for spoilage of, in storage. H. J. Smith and J. Paul Bartz.....	393
Observations on nutrition. F. L. Dunlap.....	289
Observations on the determination of H-ion concentration of dough and on the relation of H-ion to flour grade. P. Halton and E. A. Fisher.....	1
Pastry flour, Viscosity test for evaluating. Geo. L. Alexander.....	169
Peptization of flour protein and loaf volume. R. H. Harris.....	154
Photochemical action, a cause of rancidity. Mayne R. Coe and J. A. LeClerc.....	519
Phosphatides in relation to flour strength. C. O. Swanson and E. H. Kroeker.....	22
Pie flour, Tests for. C. B. Kress.....	411
Plasticity variations in dough as influenced by	
extended mixing. Oscar Skovholt and C. H. Bailey.....	523
salt. Oscar Skovholt and C. H. Bailey.....	523
skimmilk. Oscar Skovholt and C. H. Bailey.....	523
temperature. Oscar Skovholt and C. H. Bailey.....	523
Potassium bromate. Influence of successive increments of, on loaf volume. R. H. Harris.....	150
President, Address of. R. K. Durham.....	402
Protein content of	
flour and crumb color. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	121
flour and crumb texture. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	123
flour and loaf volume. R. H. Harris.....	150
flour and loaf volume. C. E. Rich.....	540
flour and loaf volume. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	123
flour and viscosity of flour-in-water suspensions. C. E. Rich.....	540
Protein, Conversion factor for, in cereals and bread. G. L. Teller.....	261

	Page
Proteins of wheat bran. G. L. Teller and W. Kedzie Teller.....	570
Punching of doughs and fermentation tolerance. C. O. Swanson and E. H. Kroeker.....	141
gas production. P. Halton and E. A. Fisher.....	40
hydrogen-ion concentration. P. Halton and E. A. Fisher.....	42
Quinhydrone, Use of, in determining pH of doughs. P. Halton and E. A. Fisher.....	2, 3, 4
Rancidity Chemical study of. H. O. Triebold and C. H. Bailey.....	50
Means of retarding. Mayne R. Coe and J. A. LeClerc.....	519
Reducing sugars in flour. M. J. Blish, and R. M. Sandstedt and G. R. Astleford.....	383
Registration at eighteenth annual convention, Detroit.....	446, 447
Relation between total protein, peptizable protein, and loaf volume as obtained by successive increments of $KBrO_3$. R. H. Harris.....	147
Relationship between viscosity, total protein content, ash content, and baking strength of experimentally and commercially milled flours. C. E. Rich....	535
Report of A. A. C. C. baking research fellowship. P. P. Merritt, M. J. Blish and R. M. Sandstedt.....	175
Cereal section of the Association of Official Agricultural Chemists, 1931. J. A. LeClerc.....	83
Editor-in-chief of Cereal Chemistry for 1931. D. A. Coleman.....	439
Managing-editor of Cereal Chemistry for 1931. C. C. Fifield.....	440
Secretary-Treasurer. M. D. Mize.....	325
Reports of committees of A. A. C. C. Auditing committee. V. E. Fisher, Chairman.....	443
Cake and biscuit flour testing committee. Mary M. Brooke, Chairman....	406
Employment committee. C. B. Morison, Chairman.....	441
Executive committee. L. D. Whiting, Chairman.....	440
History committee. R. W. Mitchell, Chairman.....	441
Membership committee. L. E. Leatherock, Chairman.....	442
Methods of analysis committee. C. E. Mangels, Chairman.....	429
Resolutions committee. Paul Logue, Chairman.....	442
Rosenwald Museum of Science committee. F. L. Dunlap, Chairman.....	441
Standard baking test committee. D. A. Coleman, Chairman.....	404
Rice brown, Chemical composition of. J. A. LeClerc.....	600
glutenous, Chemical composition of. J. A. LeClerc.....	601
Loss of constituents of, due to polishing. J. A. LeClerc.....	601
polished, Constituents of. J. A. LeClerc.....	601
polished, Cooking quality of. Mabel C. Stienbarger.....	317
rough, Chemical composition of. J. A. LeClerc.....	600
Rich, C. E. A note on the use of selenium oxychloride as a catalyst in the determination of nitrogen by the Kjeldahl method.....	118
Relationship between viscosity, total protein content, ash content, and baking strength of experimentally and commercially milled flours....	535
Rope, Determination of, in bread. H. H. Bunzell and Marjorie Kenyon.....	163
Sako, Tsunenaka and Yoshitaka Hashitani. On the vitamin B content of bread	107
Salt Effect of, on gas production in dough. W. L. Heald.....	609
Effect of, on plasticity of dough. Oscar Skovholt and C. H. Bailey.....	523
Sandstedt, R. M. Selenium as a catalyst in Kjeldahl digestion with natural gas heat.....	156
G. R. Astleford and M. J. Blish. Sugars, diastatic activity, and gassing powers in flour.....	378
M. J. Blish and P. P. Merritt. Report of the activities of the A. A. C. C. baking research fellow.....	175
Schulerud, A. The determination of acidity in flour.....	128
Schultz, A. S. and Quick Landis. Hydrolysis of starch in bread by flour and malt amylase.....	305
Scoring cake. R. A. Barackman.....	409

	Page
Selenium as a catalyst in Kjeldahl digestion with natural gas heat. R. M. Sandstedt	156
Selenium oxychloride as a catalyst in the determination of nitrogen by the Kjeldahl method. C. E. Rich	118
Self-rising flours	
Baking test for. L. D. Whiting	416
Gasometric tests for. L. D. Whiting	417
Sherwood, R. C., C. H. Bailey and A. E. Treloar. Some relationships involving crumb texture and color	121
Significance of H-ion concentration in panary fermentation. P. Halton and E. A. Fisher	34
Skimmilk, Effect of, on properties of dough as measured by the farinograph. Oscar Skovholt and C. H. Bailey	523
Skovholt, Oscar and C. H. Bailey. Effect of temperature and the inclusion of dry skimmilk upon the properties of dough as measured by the farinograph	523
Smith, E. E., Chairman, Report of subcommittee on hydrogen-ion concentration with special reference to effect of flour bleach	424
Smith, H. J. and J. Paul Bartz. Heating of feed grains	393
Soft winter wheat, Program for improvement of, in Ohio. E. G. Bayfield	322
Staling of bread, Constituents retarding. L. H. Bailey	65
Standard experimental baking test, Factors influencing. P. P. Merritt, M. J. Blish and R. M. Sandstedt	175
Baking pans, size, shape and composition of	206
Dough mixing apparatus	178
Dough molding and punching devices	179, 184, 223
Fermentation bowls, size, shape, and handling of	205
Modifications of baking test formula	213, 225
Molding of dough, hand versus machine	184, 223
Oven temperatures, effect of top heat	221, 226
Punching of doughs, hand versus machine	191
Salt effects	224
Water absorption of dough, optimum conditions for	202
Yeast handling	220
Starch	
Effect of, in bread doughs on fermentation tolerance. C. O. Swanson and E. H. Kroeker	141
Hydrolysis of, in bread by flour and malt amylase. A. S. Schultz and Quick Landis	305
Stateler, E. S. Book review. The law of patents for chemists, by Joseph Rossman	328
Statistics in the service of cereal chemistry. Alan E. Treloar	573
Stienbarger, Mabel C. Cooking qualities of domestic rices	317
Studies with the farinograph for predicting the most suitable types of American export wheats and flours for mixing with European soft wheats and flours. C. W. Brabender	617
Sugars	
diastatic activity, and gassing power in flour. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	378
Effect of sucrose on pH and CO ₂ development in bread dough fermentation. P. Halton and E. A. Fisher	38
Effect of, in bread doughs on fermentation by-products. M. J. Blish and R. C. Hughes	331
Effect of, in bread doughs on fermentation tolerance. C. O. Swanson and E. H. Kroeker	142
Effect of, in bread doughs on fermentation tolerance. M. J. Blish and R. C. Hughes	331
Sugars in flour	
Maltose. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	379
Sucrose. M. J. Blish, R. M. Sandstedt, and G. R. Astleford	385
Swanson, C. O. and E. H. Kroeker	
Some factors in fermentation tolerance	137
Testing wheat varieties for milling and baking qualities	10

	Page
Teller, G. L.	
Non-protein nitrogen compounds in cereals and their relation to the nitrogen factor for proteins in cereals and bread.....	261
and W. Kedzie Teller. Proteins of the wheat bran.....	560
Temperature and heating of feed grains. H. J. Smith and J. Paul Bartz.....	393
Testing wheats for milling and baking quality. C. O. Swanson and E. H. Kroeker.....	10
Texture of crumb	
and color of crumb. A. E. Treloar, R. C. Sherwood and C. H. Bailey....	124
and loaf volume. A. E. Treloar, R. C. Sherwood and C. H. Bailey.....	124
and protein in flour. A. E. Treloar, R. C. Sherwood and C. H. Bailey....	124
Treloar, Alan E.	
Book review, The methods of statistics, by L. H. C. Tippett.....	638
Statistics in the service of cereal chemistry.....	573
R. C. Sherwood and C. H. Bailey. Some relationships involving crumb texture and color.....	121
Triebold, H. O. and C. H. Bailey. A chemical study of rancidity, I, II.....	50, 91
Variation in weight of given volume of different flours as influenced by methods of handling. Emily Grewe.....	311
different classes of flours. Emily Grewe.....	531
milling, blending, handling, and time of storage. Emily Grewe.....	628
Viscosity	
and loaf volume. C. E. Rich.....	535
and protein content of flour. W. H. Hanson.....	376
Effect of flour bleaching on. W. H. Hanson.....	358
in relation to cake flour quality. E. E. Smith.....	424
of flour grades, Factors influencing. C. E. Rich.....	535
of flour-in-water suspensions. C. E. Rich.....	535
Report of subcommittee on. G. L. Alexander.....	428
Vitamin B content of bread. Yoshitaka Hashitani and Tsunenaka Sako.....	107
Effect of heat on.....	115
Effect of hydrogen-ion concentration on.....	115
Water absorption by wheat	
Influence of temperature of, on. C. W. Fraser and W. L. Haley.....	46, 48
Influence of time of immersion on. C. W. Fraser and W. L. Haley.....	46, 48
Influence of scouring on. C. W. Fraser and W. L. Haley.....	47
Influence of size of kernel on. C. W. Fraser and W. L. Haley.....	47
Influence of variety on. C. W. Fraser and W. L. Haley.....	46
Wattmeter, Use of, in testing wheat varieties. C. O. Swanson and E. H. Kroeker.....	22
Whitcomb, W. O. and Wm. McK. Martin. Physical and chemical properties of ether-soluble constituents of wheat flour in relation to baking quality.....	275
Whiting, L. D.	
Chairman, Report of executive committee.....	440
Chairman, Report of the subcommittee on tests for biscuit and self-rising flours.....	414
Ziegler, E.	
Book review. Alcoholic fermentation, by A. Harden.....	174
Book review. Melchemie, by K. Mohs.....	174

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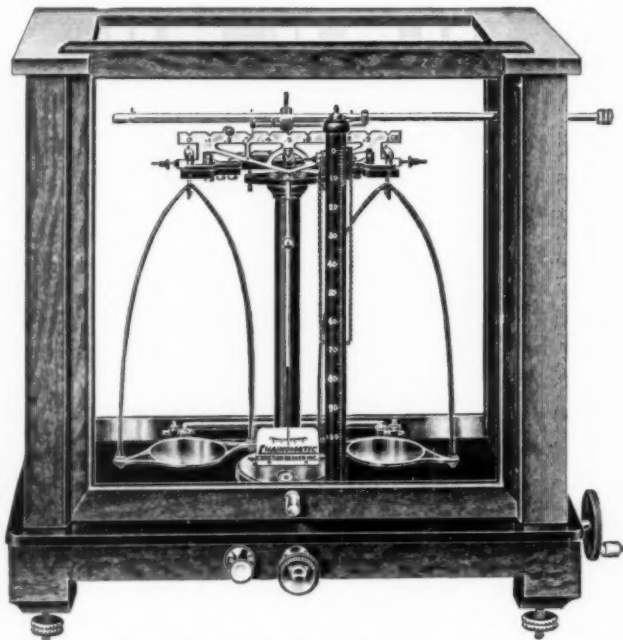
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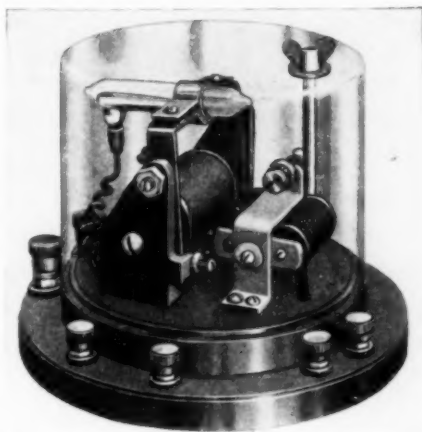
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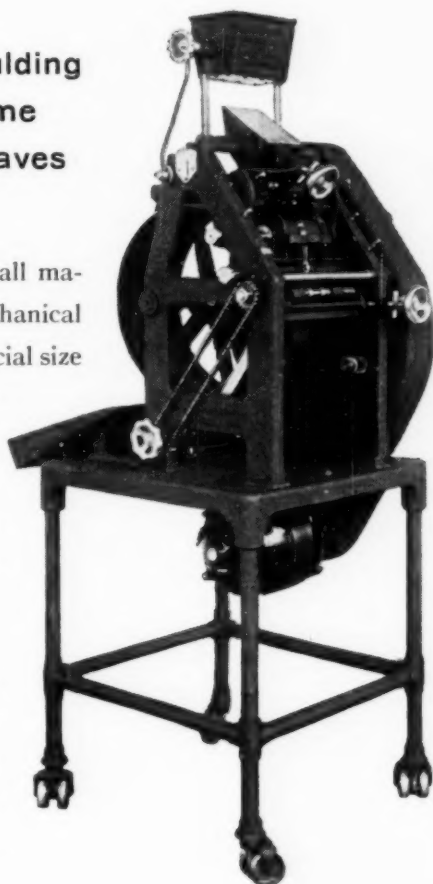
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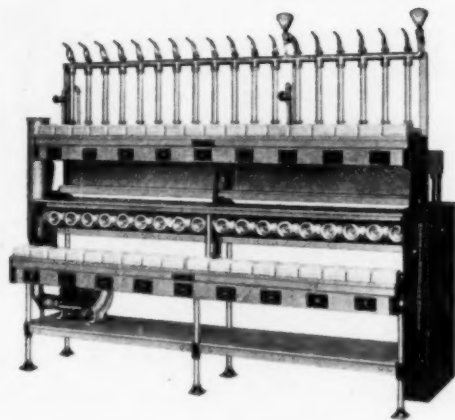
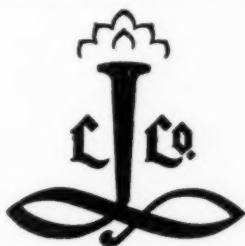
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